Burke
09/040485

FILE 'REGISTRY' ENTERED AT 15:12:59 ON 22 OCT 1999
E "ASPARTYL-.BETA.-HYDROXYLASE"/CN 5
E "HUMAN ASPARTYL-.BETA.-HYDROXYLASE"/CN 5
E "HYDROXYLASE, ASPARTYL-.BETA."/CN 5

-Key terms

FILE 'CAPLUS' ENTERED AT 15:13:58 ON 22 OCT 1999
L1 14 SEA ABB=ON PLU=ON ASPARTYL(S)HYDROXYLASE

FILE 'REGISTRY' ENTERED AT 15:15:41 ON 22 OCT 1999
E "ASPARTYL .BETA.-HYDROXYLASE"/CN 5
L2 1 SEA ABB=ON PLU=ON "ASPARTYL .BETA.-HYDROXYLASE"/CN

FILE 'CAPLUS' ENTERED AT 15:17:50 ON 22 OCT 1999
L3 33 SEA ABB=ON PLU=ON (ASPARTYL OR HA!H)(S)(HYDROXYLASE OR HYDROLASE)
L4 20 SEA ABB=ON PLU=ON L3(S)BETA
L5 11 SEA ABB=ON PLU=ON L2
L6 20 SEA ABB=ON PLU=ON L4 OR L5

L6 ANSWER 1 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:614146 CAPLUS
TITLE: cloning, cDNA sequence and mapping of a human gene encoding labyrinthin, a cancer-associated antigen
INVENTOR(S): Radosevich, James A.
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9947683	A1	19990923	WO 1999-US5365	19990311

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1998-40485 19980317
AB A cDNA mol. that encodes a protein designated Labyrinthin (Lab), the adenocarcinoma-assocd. antigen recognized by the monoclonal antibody (MCA) 44-3A6, is isolated and its nucleotide sequence is detd.

Searcher : Shears 308-4994

Labyrinthin has about a 99.6% homol. with an internal segment of the protein coding region for human **aspartyl .beta.-hydrolase** (**HAAH**). The protein, or peptides derived from the protein, are markers useful to define novel classes of cancers. Diagnostic assays for these cancers use antibodies to Lab or nucleotide probes that hybridize with the lab gene or a fragment therefrom. Vaccines useful either to prevent recurrence of cancers in subjects who test pos. for Lab (or lab), or to prevent initial occurrence of cancer, use proteins or peptides derived from Lab. Expression of Lab via immunogenic assays is used to monitor effects of cancer treatments. Antisense mols. against lab are used in treatments. Sense mols. of lab are used to restore lost lab function in diseased normal cells, for example, gland cells.

IT Human chromosome 2
(2q12-14, gene lab mapped to; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Protein motifs
(Ca++ binding, membrane anchoring; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Genes (animal)
RL: BOC (Biological occurrence); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(Lab, for labyrinthin; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Gene expression
(Lab, in cancer cells, monitoring effects of cancer treatments by assaying of; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Probes (nucleic acid)
RL: ARG (Analytical reagent use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(Lab, labeled, cancer diagnosis using; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Monoclonal antibodies
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(MCA 44-3A6, labyrinthin detected by; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Vaccines
(antigen labyrinthin as; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Antitumor agents
Cancer diagnosis
Molecular cloning
(cloning, cDNA sequence and mapping of human gene encoding

labyrinthin, cancer-assocd. antigen)

IT Antibodies
RL: ARU (Analytical role, unclassified); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Antisense DNA
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(for labyrinthin gene; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT cDNA sequences
(for labyrinthin of human; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Adenocarcinoma
(labyrinthin assocd. with; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT A549 cell
(labyrinthin gene cloned from; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Antigens
Proteins (specific proteins and subclasses)
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(labyrinthin; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Genetic mapping
(of Lab gene to human chromosome 2; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Protein sequences
(of labyrinthin of human; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT Epitope mapping
(using antibody MCA 44-3A6; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT 244082-46-0P 244082-53-9P 244082-56-2P 244082-64-2P 244082-73-3P 244082-84-6P 244144-71-6DP, Labyrinthin (human), subfragments are claimed
RL: BOC (Biological occurrence); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(amino acid sequence; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

IT 244144-70-5D, DNA (human labyrinthin cDNA plus flanks), subfragments are claimed
RL: BOC (Biological occurrence); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); USES

(Uses)
(nucleotide sequence; cloning, cDNA sequence and mapping of human gene encoding labyrinthin, cancer-assocd. antigen)

L6 ANSWER 2 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:464098 CAPLUS
DOCUMENT NUMBER: 131:99269
TITLE: X-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes
INVENTOR(S): Schofield, Christopher Joseph; Baldwin, Jack Edward; Roach, Peter L.; Lloyd, Matthew D.; Harlos, Karl; Andersson, Inger; Hajdu, Janos; Terwisscha van Scheltinga, Anke S.; Valegard, Karin; Ramaswamy, S.
PATENT ASSIGNEE(S): Isis Innovation Limited, UK
SOURCE: PCT Int. Appl., 178 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9933994	A1	19990708	WO 1998-GB3860	19981224
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: GB 1997-27370 19971224
GB 1998-13644 19980624

AB The three-dimensional crystal structure(s) of deacetoxycephalosporin C synthase (DAOCS) alone and complexed with Fe2+ and 2-oxoglutarate are described. The x-ray coordinates provide precise 3-dimensional information of amino acids within the structure of DAOCS. Information from the structures is used to modify enzymes of the cephalosporin biosynthesis pathway including DAOCS and deacetylcephalosporin C synthase DAOC/DACS, such that they accept unnatural substrates (e.g. penicillins G, V) in order to improve the prodn. of .beta.-lactam antibiotics. The structures may be used to predict the structures of other 2-oxoglutarate-dependent enzymes, thereby allowing the design of inhibitors, and new catalysts for the prodn. of e.g. oxidized amino acids/peptides. Specific

modifications of amino acid residues are proposed and exemplified.

IT Enzymes, biological studies
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(2-oxoglutarate-dependent; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT Herbicides
(design of new herbicides to overcome resistance; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT Arthritis
Fibrosis
Genetic diseases
Liver cirrhosis
(treatment of; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT Conformation (protein)
Crystal structure
Protein engineering
Streptomyces clavuligerus
(x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT .beta.-Lactams
RL: BPN (Biosynthetic preparation); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT 61-33-6, Penicillin G, biological studies 87-08-1, Penicillin V 551-16-6, 6-Aminopenicillanic acid 26787-78-0, Amoxycillin
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(altered metab. of; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT 230298-54-1
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT 1406-05-9P, Penicillin 11111-12-9P, Cephalosporin
RL: BPN (Biosynthetic preparation); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)

(biosynthesis of; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT 150-86-7, Phytol 83200-96-8, Carbapenem
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(oxygenase in biosynthesis of; x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

IT 328-50-7D, 2-Oxoglutaric acid, complex with iron(2+) and deacetoxycephalosporin C synthetase 7439-89-6D, Iron, complex with deacetoxycephalosporin C synthetase and oxoglutarate 9028-06-2, Proline hydroxylase 9029-72-5, p-Hydroxyphenylpyruvate dioxygenase 9045-31-2, .gamma.-Butyrobetaine hydroxylase 9059-25-0, Lysyl hydroxylase 15438-31-0D, Iron(2+), complex with deacetoxycephalosporin C synthetase and oxoglutarate, biological studies 57219-73-5, ACV synthetase 57827-76-6, Deacetylcephalosporin C acetyltransferase 63551-75-7, Prolyl 3-hydroxylase 75991-43-4, Flavanone 3.beta.-hydroxylase 78642-31-6, Isopenicillin N synthetase 85746-10-7, Deacetoxycephalosporin C synthetase 85746-10-7D, Deacetoxycephalosporin C synthetase, complex with iron(2+) and 2-oxoglutarate 98668-53-2 103865-33-4, Hyoscyamine 6.beta.-hydroxylase **122544-66-5**, **Aspartyl .beta.-hydroxylase** 122799-56-8, Clavaminate synthase 138263-93-1, Gibberellin 20 oxidase 138263-98-6 138757-25-2 229963-33-1, Cephamycin C 7.alpha.-oxygenase
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(x-ray structure of Streptomyces clavuligerus deacetoxycephalosporin C synthase and its use in designing modified related enzymes)

L6 ANSWER 3 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:455954 CAPLUS
DOCUMENT NUMBER: 131:210733
TITLE: Development of a carbon dioxide-capture assay in microtiter plate for **aspartyl-.beta.-hydroxylase**
AUTHOR(S): Zhang, Ji-Hu; Qi, Richard C. C.; Chen, Tongming; Chung, Thomas D. Y.; Stern, Andrew M.; Hollis, Gregory F.; Copeland, Robert A.; Oldenburg, Kevin R.
CORPORATE SOURCE: DuPont Pharmaceuticals Research Laboratories, Leads Discovery, DuPont Pharmaceuticals Company, Wilmington, DE, 19880-0400, USA
SOURCE: Anal. Biochem. (1999), 271(2), 137-142
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal

LANGUAGE: English

AB CO2-capture methods have been used for assaying many decarboxylating enzymes including hydroxylation-coupled decarboxylation reactions. The traditional CO2-capture method involves performing the reaction in capped tubes and radiometric measurement of trapped 14CO2 by scintillation counting. In this report, a 14CO2-capture method in a 96-well microtiter plate format has been developed and a phosphor imaging system has been employed for sample measurement. The new assay method has been used successfully to assay **aspartyl -.beta.-hydroxylase** activity in microtiter plate format. The results obtained here compare favorably with those obtained from the traditional tube method. The method is sensitive, suitable for high throughput, and generally applicable to many CO2-releasing enzyme assays. (c) 1999 Academic Press.

IT Microtiter plates
(development of a carbon dioxide-capture assay in microtiter plate for **aspartyl-.beta.-hydroxylase**
)

IT 124-38-9, Carbon dioxide, analysis **122544-66-5**, **Aspartyl-.beta.-hydroxylase**
RL: ANT (Analyte); ANST (Analytical study)
(development of a carbon dioxide-capture assay in microtiter plate for **aspartyl-.beta.-hydroxylase**
)

L6 ANSWER 4 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:144678 CAPLUS
DOCUMENT NUMBER: 131:29010
TITLE: A natural grouping of motifs with an aspartate or asparagine residue forming two hydrogen bonds to residues ahead in sequence: Their occurrence at .alpha.-helical N termini and in other situations
AUTHOR(S): Wan, Wai-Yan; Milner-White, E. James
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow, G12 8QQ, UK
SOURCE: J. Mol. Biol. (1999), 286(5), 1633-1649
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Examn. of the ways side-chain carboxylate and amide groups in high-resoln. protein crystal structures form H-bonds with main-chain atoms reveals that the most common category is a 2-H-bond 4-5-residue motif with Asp or Asn (Asx) at the 1st residue, for which the authors propose the name Asx-motif. Similar motifs with Glu or Gln residues at that position are rare. Asx-motifs occur typically as (1) a common feature of the N-termini of .alpha.-helixes called the Asx N-cap motif; (2) an independent

motif, usually a .beta.-turn with appropriately H-bonded Asx as the 1st residue; and (3) a motif incorporated in a .beta.-bulge loop. Asx-motifs are common, there being just under 2.5 in an av.-sized protein subunit; of these, .apprx.55% are Asx N-cap motifs. Because they occur often in many situations, it seems that these motifs have an inherent propensity to form on their own rather than just being a feature stabilized at the end of a helix. Asx-motifs also occur in functionally interesting situations in **aspartyl** proteases, citrate synthase, EF hands, Hbs, lipocalins, glutathione reductase, and the .alpha./.**beta**. **hydrolases**. (c) 1999 Academic Press.

IT Protein motifs
(Asx-motifs; a natural grouping of Asx-motifs with an Asp or Asn residue forming 2 hydrogen bonds to residues ahead in sequence and their occurrence at .alpha.-helical N-termini and in other situations)

IT Hydrogen bond
.alpha.-Helix (protein conformation)
(a natural grouping of Asx-motifs with an Asp or Asn residue forming 2 hydrogen bonds to residues ahead in sequence and their occurrence at .alpha.-helical N-termini and in other situations)

IT 70-47-3, L-Asparagine, biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(a natural grouping of Asx-motifs with an Asp or Asn residue forming 2 hydrogen bonds to residues ahead in sequence and their occurrence at .alpha.-helical N-termini and in other situations)

IT 56-84-8, L-Aspartic acid, biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(a natural grouping of motifs with an Asp or Asn residue forming 2 hydrogen bonds to residues ahead in sequence and their occurrence at .alpha.-helical N-termini and in other situations)

L6 ANSWER 5 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:672033 CAPLUS
DOCUMENT NUMBER: 130:34892
TITLE: Glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides
AUTHOR(S): Noronkoski, Tiina; Stoineva, Ivanka B.; Ivanov, Ivailo P.; Petkov, Dimiter D.; Mononen, Ilkka
CORPORATE SOURCE: Department of Clinical Chemistry, Kuopio University Hospital, Kuopio, FIN-70211, Finland
SOURCE: J. Biol. Chem. (1998), 273(41), 26295-26297
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB .beta.-Aspartyl di- and tripeptides are common constituents of mammalian metab., but their formation and catabolism are not fully understood. In this study we provide evidence that glycosylasparaginase (aspartylglucosaminidase), an N-terminal nucleophile **hydrolase** involved in the hydrolysis of the N-glycosidic bond in glycoproteins, catalyzes the hydrolysis of .**beta.-aspartyl** peptides to form L-aspartic acid and amino acids or peptides. The enzyme also effectively catalyzes the synthesis of .beta.-aspartyl peptides by transferring the .beta.-aspartyl moiety from other .beta.-aspartyl peptides or .beta.-aspartylglycosylamine to a variety of amino acids and peptides. Furthermore, the enzyme can use L-asparagine as the .beta.-aspartyl donor in the formation of .beta.-aspartyl peptides. The data show that synthesis and degrdn. of .beta.-aspartyl peptides are new, significant functions of glycosylasparaginase and suggest that the enzyme could have an important role in the metab. of .beta.-aspartyl peptides.

IT Enzyme kinetics
Michaelis constant
(glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides)

IT Peptides, biological studies
RL: BPR (Biological process); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(.beta.-aspartyl; glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides)

IT 9075-24-5, Glycosylasparaginase 37257-23-1, .beta.-Aspartyltransferase
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides)

IT 70-47-3, L-Asparagine, biological studies 687-51-4, Leucineamide 2280-40-2, Threonineamide 2776-93-4 3790-52-1 6791-49-7, Serineamide 14540-87-5 17812-32-7 22839-61-8, .beta.-Aspartame 83345-49-7 216698-62-3 216698-67-8 216698-70-3 216698-75-8
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides)

IT 216698-77-0 216698-80-5
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(glycosylasparaginase-catalyzed synthesis and hydrolysis of .beta.-aspartyl peptides)

L6 ANSWER 6 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:579357 CAPLUS
DOCUMENT NUMBER: 129:341140

TITLE: The five cysteine residues located in the active site region of bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** are not essential for catalysis

AUTHOR(S): McGinnis, Kathleen; Ku, Gregory M.; Fu, Jeffrey; Stern, Andrew M.; Friedman, Paul A.

CORPORATE SOURCE: Merck Research Laboratories, West Point, PA, 19486, USA

SOURCE: Biochim. Biophys. Acta (1998), 1387(1-2), 454-456
CODEN: BBACAQ; ISSN: 0006-3002

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In previous chem. modification studies on bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** (I), Cys residues were implicated as crit. catalytic residues. Using site-directed mutagenesis, the 5 Cys residues located in a highly conserved region of I identified as the active site were individually mutated to Ala. Substitutions at Cys-637, Cys-644, Cys-656, Cys-681, and Cys-696 resulted in active mutant enzymes indicating that these residues are not required for catalysis.

IT Active sites (enzyme)
(five Cys residues located in the active site region of bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** are not essential for catalysis)

IT 52-90-4, L-Cysteine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(five Cys residues located in the active site region of bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** are not essential for catalysis)

IT **122544-66-5, Aspartyl** (asparaginyl) **.beta.-hydroxylase**
RL: PRP (Properties)
(five Cys residues located in the active site region of bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** are not essential for catalysis)

L6 ANSWER 7 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:579555 CAPLUS

DOCUMENT NUMBER: 125:272192

TITLE: Overexpression of human **aspartyl** (asparaginyl)**.beta.-hydroxylase** in hepatocellular carcinoma and cholangiocarcinoma

AUTHOR(S): Lavaissiere, Laurent; Jia, Steve; Nishiyama, Masaki; de la Monte, Suzanne; Stern, Andrew M.; Wands, Jack R.; Friedman, Paul A.

CORPORATE SOURCE: Molecular Hepatology Lab., Massachusetts General Hospital Cancer Center, Charlestown, MA, 02129, USA
SOURCE: J. Clin. Invest. (1996), 98(6), 1313-1323
CODEN: JCINAO; ISSN: 0021-9738
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To characterize genes that become upregulated with malignant transformation of human hepatocytes, a library of monoclonal antibodies was produced against the FOCUS hepatocellular carcinoma cell line. Antibody FB-50 reacted with an antigen that was highly expressed in 4 of 10 primary hepatocellular carcinomas, in all 20 cholangiocarcinomas the authors studied, and in a variety of transformed cell lines. This antigen was also highly expressed in neoplastic epithelial cells of breast and colon carcinomas in contrast to its low level of expression in normal hepatocytes and in non-neoplastic epithelial cells. Among the normal adult tissues studied, high levels were obsd. only in proliferating trophoblastic cells of the placenta and in adrenal glands. A 636-bp partial cDNA, isolated from a .gamma.GT11 expression library generated with HepG2 human hepatoblastoma cells, and a complete cDNA, generated by reverse transcriptase-PCR, identified the antigen as the human form of **aspartyl**(asparaginyl)**.beta.**-**hydroxylase**. This enzyme catalyzes post-translational hydroxylation of .beta. carbons of specific aspartyl and asparaginyl residues in EGF-like domains of certain proteins. Analyses of exts. prepd. from several human tumor cell lines compared to their normal tissue counterparts indicate that the increase in hydroxylase, .apprx. 10-fold, is controlled at the level of transcription and the protein is expressed in an enzymically active form. In similar analyses, comparing hepatocellular carcinomas to adjacent uninvolved liver from five patients, enzymic activity was much higher in the tumor tissue from the four patients whose immunoblots revaled increased hydroxylase protein in the malignant tissue. EGF repeats in the extracellular domain of Notch or its homologs contain the consensus sequence for hydroxylation. Deletion mutants lacking this domain are gain-of-function mutants, suggesting that the domain modulates signal transduction by the cytoplasmic domain. While the function imparted by .beta. hydroxylation is unknown, the studies raise the possibility that .beta. hydroxylation is regulated in proteins like the mammalian Notch homologs, whose cytoplasmic domains have been shown to be oncogenic.

IT Adrenal gland
Placenta
(**aspartyl**(asparaginyl)**.beta.**-**hydroxylase** expression in human)

IT Transformation, neoplastic
(cDNA sequence for **aspartyl**(asparaginyl)**.beta.**-**hydroxylase** of human and overexpression in

hepatocellular carcinoma and cholangiocarcinoma)
IT Protein sequences
(of **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human)
IT Intestine, neoplasm
(colon, carcinoma, overexpression of human **aspartyl**(asparaginyl)**.beta.-hydroxylase** in)
IT Deoxyribonucleic acid sequences
(complementary, for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human)
IT Liver, neoplasm
(hepatoma, cDNA sequence for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human and overexpression in hepatocellular carcinoma and cholangiocarcinoma)
IT Mammary gland
(neoplasm, carcinoma, overexpression of human **aspartyl**(asparaginyl)**.beta.-hydroxylase** in)
IT Biliary tract
(neoplasm, cholangioma, cDNA sequence for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human and overexpression in hepatocellular carcinoma and cholangiocarcinoma)
IT 182441-85-6
RL: PRP (Properties)
(amino acid sequence; cDNA sequence for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human and overexpression in hepatocellular carcinoma and cholangiocarcinoma)
IT **122544-66-5**, **Aspartyl**(asparaginyl) **.beta.-hydroxylase**
RL: PRP (Properties)
(cDNA sequence for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human and overexpression in hepatocellular carcinoma and cholangiocarcinoma)
IT 182441-84-5
RL: PRP (Properties)
(nucleotide sequence; cDNA sequence for **aspartyl**(asparaginyl)**.beta.-hydroxylase** of human and overexpression in hepatocellular carcinoma and cholangiocarcinoma)

L6 ANSWER 8 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1996:156925 CAPLUS
DOCUMENT NUMBER: 124:224840
TITLE: Site-directed mutagenesis of residues in a conserved region of bovine **aspartyl**(asparaginyl) **.beta.-hydroxylase**: Evidence that histidine 675 has a role in binding Fe2+
Searcher : Shears 308-4994

AUTHOR(S): McGinnis, Kathleen; Ku, Gregory M.; VanDusen, William J.; Fu, Jeffrey; Garsky, Victor; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Merck Research Laboratories, West Point, PA, 19486, USA
SOURCE: Biochemistry (1996), 35(13), 3957-62
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The roles in catalysis of several residues in bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** (I) that are located in a region of homol. among .alpha.-ketoglutarate-dependent dioxygenases were investigated using site-directed mutagenesis. Previous studies showed that when His-675, an invariant residue located in this highly conserved region, was mutated to an Ala residue, no enzymic activity was detected. A more extensive site-directed mutagenesis study at position 675 was undertaken to define the catalytic role of this essential His residue. The partial I activity obsd. with some amino acid replacements for His-675 correlated with the potential to coordinate metal cations and not with size, charge, or hydrophobic character. Furthermore, the increase in Km for Fe2+ obsd. with the H675D and H675E mutants could account for their partial activities relative to wild-type I. No significant changes in Km for .alpha.-ketoglutarate (at satg. Fe2+) or Vmax were obsd. for these mutants. These results support the conclusion that His-675 is specifically involved in Fe2+ coordination. Further site-directed mutagenesis of other highly conserved residues in the vicinity of position 675 demonstrated the importance of this region of homol. in catalysis for I and, by analogy, other .alpha.-ketoglutarate-dependent dioxygenases.

IT Michaelis constant
(of **aspartyl** (asparaginyl) **.beta.-hydroxylase** wild-type and mutant forms from bovine liver)

IT Liver
(role of His-675 in binding Fe2+ in bovine liver **aspartyl** (asparaginyl) **.beta.-hydroxylase**)

IT Enzyme functional sites
(cation-binding, role of His-675 in binding Fe2+ in bovine liver **aspartyl** (asparaginyl) **.beta.-hydroxylase**)

IT 71-00-1, Histidine, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(675; role of His-675 in binding Fe2+ in bovine liver **aspartyl** (asparaginyl) **.beta.-hydroxylase**)

IT 7439-89-6, Iron, biological studies
RL: BPR (Biological process); BIOL (Biological study); PROC

(Process)
(role of His-675 in binding Fe2+ in bovine liver **aspartyl** (asparaginyl) **.beta.-hydroxylase**)

IT **122544-66-5**, **Aspartyl** (asparaginyl) **.beta.-hydroxylase**
RL: BPR (Biological process); PRP (Properties); BIOL (Biological study); PROC (Process)
(role of His-675 in binding Fe2+ in bovine liver **aspartyl** (asparaginyl) **.beta.-hydroxylase**)

L6 ANSWER 9 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1996:11683 CAPLUS
DOCUMENT NUMBER: 124:110186
TITLE: Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (Junctin) from cardiac junctional sarcoplasmic reticulum
AUTHOR(S): Jones, Larry R.; Zhang, Lin; Sanborn, Kristi; Jorgensen, Annelise O.; Kelley, Jeff
CORPORATE SOURCE: Sch. Med., Indiana Univ., Indianapolis, IN, 46202, USA
SOURCE: J. Biol. Chem. (1995), 270(51), 30787-96
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Previously the authors identified a protein of apparent Mr = 26,000 as the major calsequestrin binding protein in junctional sarcoplasmic reticulum vesicles isolated from cardiac and skeletal muscle (Mitchell, R. D., Simmerman, H. K. B., and Jones, L. R. (1988) J. Biol. Chem. 263, 1376-1381). Here the authors describe the purifn. and primary structure of the 26-kDa calsequestrin binding protein. The protein was purified 164-fold from cardiac microsomes and shown by immunoblotting to be highly enriched in junctional membrane subfractions. It ran as a closely spaced doublet on SDS-polyacrylamide gel electrophoresis and bound 125I-calsequestrin intensely. Cloning the cDNA predicted a protein of 210 amino acids contg. a single transmembrane domain. The protein has a short N-terminal region located in the cytoplasm, and the bulk of the mol., which is highly charged and basic, projects into the sarcoplasmic reticulum lumen. Significant homols. were found with triadin and **aspartyl .beta.-hydroxylase**, suggesting that all three proteins are members of a family of single membrane-spanning endoplasmic reticulum proteins. Immunocytochem. labeling localized the 26-kDa protein to junctional sarcoplasmic reticulum in cardiac and skeletal muscle. The same gene product was expressed in these two tissues. The calsequestrin binding activity of the 26-kDa protein combined with its codistribution with calsequestrin and ryanodine receptors strongly suggests that the protein plays an important role in the

organization and/or function of the Ca2+ release complex. Because the 26-kDa calsequestrin binding protein is an integral component of the junctional sarcoplasmic reticulum membrane in cardiac and skeletal muscle, have named it Junctin.

IT Gene, animal
RL: PRP (Properties)
(for junction; purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

IT Canis familiaris
Muscle
(purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

IT Proteins, specific or class
RL: PRP (Properties); PUR (Purification or recovery); PREP (Preparation)
(calsequestrin-binding, Junctin; purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

IT Endoplasmic reticulum
(sarcoplasmic reticulum, purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

IT 173048-63-0P
RL: PRP (Properties); PUR (Purification or recovery); PREP (Preparation)
(amino acid sequence; purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

IT 172776-50-0, Genbank U38414
RL: PRP (Properties)
(nucleotide sequence; purifn. and primary structure and immunol. characterization of 26-kDa calsequestrin binding protein (Junctin))

L6 ANSWER 10 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:286733 CAPLUS
DOCUMENT NUMBER: 122:179957
TITLE: Cloning and characterization of the human gene encoding **aspartyl .beta.-hydroxylase**
AUTHOR(S): Korioth, Frank; Gieffers, Christian; Frey, Juergen
CORPORATE SOURCE: Universitaet Bielefeld, Fakultaet fuer Chemie, Biochemie II, 33615, Bielefeld, Germany
SOURCE: Gene (1994), 150(2), 395-9
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sequence information for **aspartyl .beta.-hydroxylase** (AspH), which specifically hydroxylates one Asp or Asn residue in certain epidermal growth factor (EGF)-like domains of a no. of proteins, is so far only described for bovine species. We have isolated a 4.3-kb cDNA encoding the human AspH (hAspH) by immunoscreening of a human osteosarcoma (MG63) cDNA library in .lambda.ZAP with an antiserum raised against membrane fractions of these cells. Northern blot analyses revealed two transcripts with lengths of 2.6 and 4.3kb. The deduced amino acid (aa) sequence of this cDNA encodes a protein of 757 aa (85kDa). Comparison with the deduced bovine AspH (bAspH) aa sequence showed striking differences in the N-terminal portion of this protein. In vitro transcription and translation in the presence of canine pancreas microsomes yielded a 56-kDa protein. Western blot analyses of membrane fractions from MG63 cells with AspH-specific antibodies revealed a protein of the same Mr. These results suggest a posttranslational cleavage of the catalytic C terminus in the lumen of the endoplasmic reticulum.

IT Gene, animal
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(ASPH; DNA sequence of human gene encoding **aspartyl .beta.-hydroxylase**)

IT Protein sequences
(of human **aspartyl .beta.-hydroxylase**)

IT Deoxyribonucleic acid sequences
(complementary, DNA sequence of human gene encoding **aspartyl .beta.-hydroxylase**)

IT **122544-66-5**
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(DNA sequence of human gene encoding **aspartyl .beta.-hydroxylase**)

IT 153662-16-9
RL: PRP (Properties)
(DNA sequence of human gene encoding **aspartyl .beta.-hydroxylase**)

IT 161630-81-5
RL: PRP (Properties)
(amino acid sequence; DNA sequence of human gene encoding **aspartyl .beta.-hydroxylase**)

L6 ANSWER 11 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1994:573847 CAPLUS
DOCUMENT NUMBER: 121:173847
TITLE: A fully active catalytic domain of bovine **aspartyl** (asparaginyl) **.beta.-hydroxylase** expressed in Escherichia coli: characterization and evidence for the identification of an active-site region in

vertebrate .alpha.-ketoglutarate-dependent dioxygenases

AUTHOR(S): Jia, Steve; McGinnis, Kathleen; VanDusen, William J.; Burke, Carl J.; Kuo, Amy; Griffin, Patrick R.; Sardana, Mohinder K.; Elliston, Keith O.; Stern, Andrew M.; Friedman, Paul A.

CORPORATE SOURCE: Merck Res. Lab., West Point, PA, 19486, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(15), 7227-31
CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The .alpha.-ketoglutarate-dependent dioxygenase **aspartyl** (asparaginyl) .**beta.**-**hydroxylase** (EC 1.14.11.16) specifically hydroxylates one aspartic or asparagine residue in certain epidermal growth factor-like domains of a no. of proteins. The expression in Escherichia coli, purifn., characterization of a fully active catalytic domain, and evidence for the identification of an active-site region of this enzyme are described. Sequence alignment analyses among the vertebrate .alpha.-ketoglutarate-dependent dioxygenases and chem. modification studies were undertaken aimed at locating specific regions of 52-kDa recombinant **aspartyl** (asparaginyl) .**beta.**-**hydroxylase** involved in substrate binding and/or catalysis. Based upon these studies, an alignment of the C-terminal regions of prolyl and lysyl **hydroxylase** and of **aspartyl** (asparaginyl) . **beta.**-**hydroxylase** is proposed. When histidine-675, an invariant residue located in a region of homol. within this alignment, was mutated to an alanine residue in **aspartyl** (asparaginyl) .**beta.**-**hydroxylase** (H675A), no enzymic activity was detected. Chem. modification studies show that the wild-type protein is protected from iodo[14C]acetamide labeling by Fe2+/.alpha.-ketoglutarate whereas the H675A mutant protein is not, suggesting that this mutant does not bind Fe2+/.alpha.-ketoglutarate.

IT Enzyme functional sites
(of aspartyl (asparaginyl) hydroxylase, essential histidine residue in, other vertebrate ketoglutarate-dependent dioxygenases in relation to)

IT **122544-66-5**, E.C. 1.14.11.16
RL: BIOL (Biological study)
(active site region and essential histidine residue of recombinant form of, of cattle, other ketoglutarate-dependent dioxygenases in relation to)

IT 328-50-7, .alpha.-Ketoglutaric acid 7439-89-6, Iron, biological studies
RL: BIOL (Biological study)
(aspartyl (asparaginyl) hydroxylase interaction with, histidine-675 role in)

IT 71-00-1, Histidine, properties
RL: PRP (Properties)
(essential, of aspartyl (asparaginyl) hydroxylase active site region)
IT 37292-90-3, Dioxygenase
RL: BIOL (Biological study)
(.alpha.-ketoglutarate-dependent, multiple forms of, active site region of)

L6 ANSWER 12 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1993:229018 CAPLUS
DOCUMENT NUMBER: 118:229018
TITLE: cDNA cloning and expression of bovine **aspartyl** (asparaginyl) .**beta**.-**hydroxylase**
AUTHOR(S): Jia, Steve; VanDusen, William J.; Diehl, Ronald E.; Kohl, Nancy E.; Dixon, Richard A. F.; Elliston, Keith O.; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Merck Sharp Dohme Res. Lab., West Point, PA, 19486, USA
SOURCE: J. Biol. Chem. (1992), 267(20), 14322-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Aspartyl** (asparaginyl) .**beta**.-**hydroxylase** which specifically hydroxylates 1 Asp or Asn residue in certain epidermal growth factor-like domains of a no. of proteins, has been previously purified to apparent homogeneity from detergent-solubilized bovine liver microsomes (Wang, Q., et al., 1991). Three oligonucleotides, corresponding to three amino acid sequences of the purified hydroxylase, were used to screen bovine cDNA libraries. Several overlapping pos. cDNA clones contg. a full length open reading frame of 754 amino acids encoding a 85-kDa protein were isolated, and a cDNA, contg. the full length open reading frame, was constructed from two of these clones. The resulting clone was then transcribed and translated in vitro to produce recombinant protein which possessed Asp .beta.-hydroxylase activity. These results constitute proof that the protein purified from bovine liver is an Asp .beta.-hydroxylase. Comparisons of deduced amino acid sequences of two other .alpha.-ketoglutarate-dependent dioxygenases, prolyl-4-hydroxylase and lysyl hydroxylase, with that of Asp .beta.-hydroxylase showed no significant homologies. Indeed, Asp .beta.-hydroxylase appears to be unique as no striking homol. was found with known protein sequences. Furthermore, structural predictions derived from the deduced amino acid sequence are in accord with earlier Stokes' radius and sedimentation coeff. detns. of the enzyme, suggesting that the enzyme contains a relatively compact carboxyl-terminal catalytic

domain and an extended amino terminus. This amino-terminal region has a potential transmembrane type II signal-anchor domain that could direct that catalytic domain into the lumen of the endoplasmic reticulum.

IT Cattle
(**aspartyl** (asparaginyl) **.beta.-hydroxylase** of, amino acid sequence and expression of)

IT Protein sequences
(of **aspartyl** (asparaginyl) **.beta.-hydroxylase**, of cattle)

IT Deoxyribonucleic acid sequences
(complementary, for **aspartyl** (asparaginyl) **.beta.-hydroxylase**, of cattle)

IT 147605-36-5
RL: BIOL (Biological study); PRP (Properties)
(amino acid sequence of, complete)

IT 141161-88-8, GenBank M91213
RL: BIOL (Biological study); PRP (Properties)
(nucleotide sequence of)

L6 ANSWER 13 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1993:228763 CAPLUS
DOCUMENT NUMBER: 118:228763
TITLE: Invertebrate **aspartyl**/asparaginyl **.beta.-hydroxylase**: potential modification of endogenous epidermal growth factor-like modules
AUTHOR(S): Monkovic, Don D.; VanDusen, William J.; Petroski, Christopher J.; Garsky, Victor M.; Sardana, Mohinder K.; Zavodszky, Peter; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Merck Res. Lab., West Point, PA, 19486, USA
SOURCE: Biochem. Biophys. Res. Commun. (1992), 189(1), 233-41
CODEN: BBRCA9; ISSN: 0006-291X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An invertebrate .alpha.-ketoglutarate-dependent **aspartyl**/asparaginyl **.beta.-hydroxylase** (I), which posttranslationally hydroxylates specific **aspartyl** or asparginyl residues within EGF-like modules, was identified, partially purified, and characterized. Prepns. derived from 2 insect cell lines catalyzed the hydroxylation of the expected asparaginyl residue within a synthetic EGF-like module. This activity was similar to that of the purified mammalian I with respect to cofactor requirements, stereochem., and substrate sequence specificity. Furthermore, recombinant human complement C1.hivin.r, expressed in an insect cell-derived baculovirus expression system, was also hydroxylated at the expected asparaginyl

residue. Thus, these results establish the potential for invertebrate aspartyl/asparaginyl hydroxylation. Since several invertebrate proteins known to be required for proper embryonic development contain a putative consensus sequence that may be required for hydroxylation, the studies presented here provide the basis for further investigations concerned with identifying hydroxylated invertebrate proteins and detg. their physiol. function.

IT Drosophila melanogaster
Spodoptera frugiperda
(aspartyl/asparaginyl hydroxylase of, purifn. and characterization of)

IT **122544-66-5P**, **Aspartyl**/asparaginyl **.beta.-hydroxylase**
RL: PREP (Preparation)
(of Drosophila melanogaster and Spodoptera frugiperda, purifn. and characterization of)

IT 62229-50-9, EGF
RL: BIOL (Biological study)
(proteins contg. modules related to, hydroxylation of, by aspartyl/asparaginyl hydroxylase of invertebrates)

L6 ANSWER 14 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1991:626696 CAPLUS
DOCUMENT NUMBER: 115:226696
TITLE: Bovine liver **aspartyl .beta.-hydroxylase**. Purification and characterization
AUTHOR(S): Wang, Qingping; VanDusen, William J.; Petroski, Christopher J.; Garsky, Victor M.; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Merck Sharp and Dohme Res. Lab., West Point, PA, 19486, USA
SOURCE: J. Biol. Chem. (1991), 266(21), 14004-10
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The .alpha.-ketoglutarate-dependent dioxygenase, L-Asp(L-Asn)-.beta.-hydroxylase which posttranslationally hydroxylates specific aspartic acid (asparagine) residues within epidermal growth factor-like domains was purified from bovine liver and characterized. A 52-kDa and a 56-kDa species of this enzyme, which accounted for by and 30% of the total enzymic activity, resp., were purified to apparent homogeneity. Amino-terminal sequence analyses and immunoblots utilizing antisera raised to the intact 52-kDa species as well as to two complementary fragments of this species demonstrated that the 52- and 56-kDa species differ by a 22-amino acid amino-terminal extension. The remaining 10% of the purified enzymic activity could be accounted for by the presence of

immunol. related higher mol. mass forms (56-90 kDa) of L-Asp(L-Asn)-.beta.-hydroxylase. Strong evidence was obtained from the results of immunoextn. studies that L-Asp(L-Asn)-.beta.-hydroxylase can be identified with the purified proteins. Kinetic and phys. studies suggest that L-Asp(L-Asn)-.beta.-hydroxylase exists as a monomer with a compact catalytic domain and an extended protease-sensitive amino terminus whose function remains to be detd. Since the purified L-Asp(L-Asn)-.beta.-hydroxylase hydroxylated both L-Asp- and L-Asn-contg. substrates, it is possible that a single enzyme is responsible for the hydroxylation of aspartate and asparagine residues in vivo.

IT Liver, composition
(**aspartyl**(asparaginyl)-**.beta.**-**hydroxylase** of, purifn. and properties of)

IT Protein sequences
(of **aspartyl**(asparaginyl)-**.beta.**-**hydroxylase** multiple forms N-termini, of liver)

IT Michaelis constant
(of **aspartyl**(asparaginyl)-**.beta.**-**hydroxylase**, of liver)

IT 137177-63-0
RL: BIOL (Biological study); PRP (Properties)
(amino acid sequence of)

IT 7782-44-7, Oxygen, biological studies
RL: BIOL (Biological study)
(**aspartyl**(asparaginyl)-**.beta.**-**hydroxylase** of liver dependence on)

IT 9001-29-0, Blood-coagulation factor X
RL: PRP (Properties)
(first epidermal growth factor-like domain in, reaction of, with **aspartyl**(asparaginyl)-**.beta.**-**hydroxylase** of liver, kinetics of, asparagine substitution for aspartate-18 in EGF domain effect on)

IT **122544-66-5P**
RL: BIOL (Biological study); PRP (Properties); PUR (Purification or recovery); PREP (Preparation)
(of liver, purifn. and properties of)

IT 328-50-7 7439-89-6, Iron, reactions
RL: RCT (Reactant)
(reaction of, with **aspartyl**(asparaginyl)-**.beta.**-**hydroxylase** of liver, kinetics of)

L6 ANSWER 15 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1990:419921 CAPLUS
DOCUMENT NUMBER: 113:19921
TITLE: Partial purification and characterization of bovine liver **aspartyl .beta .-hydroxylase**
AUTHOR(S): Gronke, Robert S.; Welsch, Dean J.; VanDusen,
Searcher : Shears 308-4994

William J.; Garsky, Victor M.; Sardana, Mohinder K.; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Dep. Pharmacol., Merck Sharp and Dohme Res. Lab., West Point, PA, 19486, USA
SOURCE: J. Biol. Chem. (1990), 265(15), 8558-65
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In vitro hydroxylation of aspartic acid has recently been demonstrated in a synthetic peptide based on the structure of the first epidermal growth factor domain in human factor. The putative enzyme responsible for the post-translational modification, **aspartyl .beta.-hydroxylase**, has been shown to be a member of a class of 2-ketoglutarate-dependent dioxygenases, which include prolyl-4- and lysyl-**hydroxylases**. In the present study, the solubilization with nonionic detergent of the enzyme from bovine liver microsomes and its purifn. using DEAE-cellulose followed by heparin-Sepharose are described. No addnl. detergent was required during purifn. The partially purified enzyme prepn. was found to contain no prolyl-4- or lysyl-hydroxylase activity. Using a synthetic peptide based on the structure of the epidermal growth factor-like region in human factor X as substrate, the apparent Km values for Fe and .alpha.-ketoglutarate were 3 and 5 .mu.M, resp. The enzyme hydroxylated the factor X peptide with the same stereospecificity (erythro .beta.-hydroxyaspartic acid) and occurred only at the aspartate corresponding to the position seen in vivo. Furthermore, the extent to which either peptide (factor IX or X) was hydroxylated reflected the extent of hydroxylation obsd. for both human plasma factors IX and X.

IT Liver, composition
(aspartyl hydroxylase of microsome of, purifn. and properties of)
IT Microsome
(aspartyl hydroxylase of, of liver, purifn. and properties of)
IT Michaelis constant
(of aspartyl hydroxylase, of liver microsome)
IT Conformation and Conformers
(of blood-coagulation factor X epidermal growth factor domain, of human, reaction with aspartyl hydroxylase in relation to)
IT 9001-29-0, Blood-coagulation factor X
RL: PRP (Properties)
(aspartyl hydroxylase of liver microsome reaction with epidermal growth factor domain of, of human)
IT **122544-66-5P**
RL: PREP (Preparation)
(of liver microsome, purifn. and properties of)
IT 328-50-7, .alpha.-Ketoglutaric acid 7439-89-6, Iron, reactions
RL: RCT (Reactant)
(reaction of, of aspartyl hydroxylase of liver microsome, kinetics of)

L6 ANSWER 16 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1989:529470 CAPLUS
DOCUMENT NUMBER: 111:129470
TITLE: **Aspartyl .beta.-hydroxylase**: in vitro hydroxylation of a synthetic peptide based on the structure of the first growth factor-like domain of human factor IX
AUTHOR(S): Gronke, Robert S.; VanDusen, William J.; Garsky, Victor M.; Jacobs, John W.; Sardana, Mohinder K.; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Dep. Pharmacol., Merck Sharp and Dohme Res. Lab., West Point, PA, 19486, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1989), 86(10), 3609-13
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB .beta.-Hydroxylation of aspartic acid is a post-translational modification that occurs in several vitamin K-dependent coagulation proteins. By use of a synthetic substrate comprised of the 1st epidermal growth factor-like domain in human factor IX and either mouse L-cell exts. or rat liver microsomes as the source of enzyme, in vitro aspartyl .beta.-hydroxylation was accomplished. **Aspartyl .beta.-hydroxylase** appears to require the same cofactors as known .alpha.-ketoglutarate-dependent dioxygenases. The hydroxylation reaction proceeds with the same stereospecificity and occurs only at the aspartate corresponding to the position seen in vivo. Further purifn. and characterization of this enzymic activity should now be possible.

IT Liver, composition
(aspartyl hydroxylase of microsome of, reaction requirements and properties of)
IT Microsome
(aspartyl hydroxylase of, of liver, reaction requirements and properties of)
IT 328-50-7, 2-Ketoglutaric acid 7439-89-6, Iron, biological studies
RL: BIOL (Biological study)
(aspartyl hydroxylase of microsome requirement for)
IT **122544-66-5**
RL: BIOL (Biological study)
(growth factor domain of human blood coagulation factor IX reaction with, of microsome, enzyme reaction requirements and properties in relation to)
IT 9001-28-9, Blood coagulation factor IX
RL: PRP (Properties)
(hydroxylation of growth factor domain of, of human, by aspartyl hydroxylase of microsome, requirements and properties of)

IT 121852-48-0P
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. and aspartyl hydroxylase of microsome activity with, enzyme reaction requirements and properties in relation to)

L6 ANSWER 17 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1980:213452 CAPLUS
DOCUMENT NUMBER: 92:213452
TITLE: Effects of pH treatments and deglycosylation of rabbit immunoglobulin G on the binding of C1q
AUTHOR(S): Winkelhake, Jeffrey L.; Kunicki, Thomas J.; Elcombe, Barbara M.; Aster, Richard H.
CORPORATE SOURCE: Dep. Microbiol., Med. Coll. Wisconsin, Milwaukee, WI, 53226, USA
SOURCE: J. Biol. Chem. (1980), 255(7), 2822-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The abilities of deglycosylated or pH-denatured and renatured rabbit IgG antibodies to interact with antigen and the 1st component of complement, C1q, were studied to begin probing the environmental features of Ig domains required for the 1st step in activating the classical complement pathway. Both immune complex formation and the binding of haptens, natural antigens or bivalent affinity labels were unaltered by complete removal of oligosaccharides from IgG. Antigen binding sites were similarly unaltered after acidic or basic buffer treatments followed by reneutralization. However, removal of oligosaccharides with .**beta**.-**aspartyl** N-acetylglucosaminido **hydrolase** or incubation at pH <3.5 or >10.5 (followed by neutralization to pH 7.0) irreversibly altered the abilities of rabbit IgG antibodies to interact with C1q. This lack of interaction with C1q is due to a lack of C1q binding and not due to an anticomplementary action of deglycosylated or pH shifted IgG. Inhibition of C1q binding is also correlated with an inability of several antibody populations to facilitate complement-mediated cytolysis. IgG structural alterations induced by charge perturbation and, to a minor extent, by deglycosylation could be detected by UV difference spectroscopy. Results are discussed in terms of the possible roles that Ig-linked oligosaccharides might play in facilitating IgG binding by C1q.
IT Complement
(C1q, Ig binding to, pH and deglycosylation effects on)
IT Immunoglobulins
RL: BIOL (Biological study)
(G, complement C1q binding to, pH and deglycosylation of Ig effects on)

L6 ANSWER 18 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1979:435094 CAPLUS

DOCUMENT NUMBER: 91:35094
TITLE: Quantitative determination of 4-N-2-acetamido-2-deoxy-.beta.-D-glucopyranosyl-L-asparagine in the urine of patients with aspartylglycosaminuria by gas-liquid chromatography
AUTHOR(S): Maury, Peter
CORPORATE SOURCE: Dep. Med. Chem., Univ. Helsinki, Helsinki, Finland
SOURCE: J. Lab. Clin. Med. (1979), 93(5), 718-23
CODEN: JLCMAK; ISSN: 0022-2143
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A specific and sensitive method for the quant. detn. of acetamidodeoxyglucopyranosylasparagine (GlcNAc-Asn) in the urine of patients with inherited deficiency of the lysosomal **hydrolase N-aspartyl-.beta**.-glucosaminidase is reported. The method is based on gas chromatog. assay of GlcNAc-Asn as its methylated deriv. and requires 100 .mu.L urine. The mean urinary excretion of GlcNAc-Asn in 14 patients with aspartylglycosaminuria was 0.99 mmol/24 h (range 0.15-1.88). Young patients had similar urinary levels of GlcNAc-Asn to those of the older ones when the results were calcd. on the basis of creatinine excretion. Mass fragmentog. anal. revealed the presence of min. amts. of GlcNAc-Asn in normal urine also. In 4 of the 8 normal subjects studied, a rough quant. estn. was feasible; the urinary output of GlcNAc-Asn in these subjects ranged from .apprx.0.001 to 0.01 mmol/24 h.

IT Urine analysis
(acetamidodeoxyglucopyranosylasparagine detn. in, by gas chromatog.)
IT 2776-93-4
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, in urine by gas chromatog. in aspartylglycosaminuria)
IT 9075-24-5
RL: ANST (Analytical study)
(genetic deficiency of, acetamidodeoxyglucopyranosylasparagine detn. in urine in)
IT 70-47-3D, N-glycosaminyl derivs.
RL: ANST (Analytical study)
(metabolic disorders, aspartylglycosaminuria, acetamidodeoxyglucopyranosylasparagine of urine in)

L6 ANSWER 19 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1974:25295 CAPLUS
DOCUMENT NUMBER: 80:25295
TITLE: Enzymes hydrolyzing L-leucyl-.beta.-naphthylamide and .alpha.-L-aspartyl-p-nitroanilide in human placenta during

development
AUTHOR(S): Warwas, M.; Dobryszycka, W.; Sward, J.
CORPORATE SOURCE: Biol. Pharm. Inst., Med. Acad., Wroclaw, Pol.
SOURCE: Enzyme (1973), 14(6), 340-7
CODEN: ENZYBT
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The activities of L-leucyl-.**beta**.-naphthylamidase (EC 3.4.1.1) and .alpha.-L-**aspartyl**-p-nitroanilide **hydrolase** of placenta and related tissues were detd. in 4 periods of the development of human placenta. Both enzymes were the most active in placentas originating from the period of growth and differentiation, i.e., between the 14th and 28th wk of gestation. Three zones of L-leucyl-.beta.-naphthylamidase were demonstrated by polyacrylamide gel electrophoresis in exts. of placenta, and 4 zones in membranes. Placenta contained 1 zone of .alpha.-L-aspartylnaphthylamidase; membranes were devoid of this activity.

IT Placenta
(aspartylnitroanilide hydrolase and leucylnaphthylamidase of)

IT 9001-61-0 9074-83-3
RL: BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(of placenta)

L6 ANSWER 20 OF 20 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1973:501928 CAPLUS
DOCUMENT NUMBER: 79:101928
TITLE: Presence of .**beta**.-**aspartyl** N-acetyl glucosamine amido **hydrolase** in mammalian spermatozoa
AUTHOR(S): Bhalla, V. K.; Tillman, W. L.; Williams, W. L.
CORPORATE SOURCE: Dep. Biochem., Univ. Georgia, Athens, Ga., USA
SOURCE: J. Reprod. Fert. (1973), 34(1), 137-9
CODEN: JRPFA4
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The title enzyme (I), a lysosomal enzyme which cleaves the linkage between aspartate and N-acetyl glucosamine was found in the exts. of squirrel-monkey, ram, and human spermatozoa. The specific activity of I in acrosomal exts. of all species was greater than that of seminal plasma. I is apparently of acrosomal origin. I from human spermatozoa was purified 30-fold by chromatog. on Sephadex G-100. The pH optimum was 7.8. Max. activity was at 70.degree.. The physiol. significance of the high temp. optimum and biol. function of this new enzyme in fertilization remain unknown.

IT Sperm
(mammalian, aspartylglucosylaminase in)

IT 9075-24-5
RL: BIOL (Biological study)

(in sperm, of mammals, properties of)

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS' ENTERED AT 15:20:26 ON 22 OCT 1999)
L7 122 S L6
L8 56 DUP REM L7 (66 DUPLICATES REMOVED)

L8 ANSWER 1 OF 56 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999165791 MEDLINE
DOCUMENT NUMBER: 99165791
TITLE: A natural grouping of motifs with an aspartate or asparagine residue forming two hydrogen bonds to residues ahead in sequence: their occurrence at alpha-helical N termini and in other situations.
AUTHOR: Wan W Y; Milner-White E J
CORPORATE SOURCE: Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Mar 12) 286 (5) 1633-49.
Journal code: J6V. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199907
ENTRY WEEK: 19990701
AB Examination of the ways side-chain carboxylate and amide groups in high-resolution protein crystal structures form hydrogen bonds with main-chain atoms reveals that the most common category is a two-hydrogen-bond four to five residue motif with an aspartate or asparagine (Asx) at the first residue, for which we propose the name Asx-motif. Similar motifs with glutamate or glutamine residues at that position are rare. Asx-motifs occur typically as (1) a common feature of the N termini of alpha-helices called the Asx N-cap motif; (2) an independent motif, usually a **beta**-turn with an appropriately hydrogen-bonded Asx as the first residue; and (3) a motif incorporated in a **beta**-bulge loop. Asx-motifs are common, there being just under two-and-a-half in an average-sized protein subunit; of these, about 55 % are Asx N-cap motifs. Because they occur often in many situations, it seems that these motifs have an inherent propensity to form on their own rather than just being a feature stabilised at the end of a helix. Asx-motifs also occur in functionally interesting situations in **aspartyl** proteases, citrate synthase, EF hands, haemoglobins, lipocalins, glutathione reductase and the alpha/**beta hydrolases**.
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L8 ANSWER 2 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1999:126720 SCISEARCH

THE GENUINE ARTICLE: 162YZ
TITLE: **Aspartyl** (asparaginyl) **beta hydroxylase** (AAH) expression marks invasiveness of primary malignant CNS neoplasms.
AUTHOR: delaMonte S M (Reprint); Gemelli B; Brown N V; Wands J R
CORPORATE SOURCE: HARVARD UNIV, MASSACHUSETTS GEN HOSP, SCH MED, BOSTON, MA
COUNTRY OF AUTHOR: USA
SOURCE: LABORATORY INVESTIGATION, (JAN 1999) Vol. 79, No. 1, pp. 1000-1000.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106.
ISSN: 0023-6837.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 0

L8 ANSWER 3 OF 56 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 1999323452 EMBASE
TITLE: Crystal structure of enteropeptidase light chain complexed with an analog of the trypsinogen activation peptide.
AUTHOR: Lu D.; Futterer K.; Korolev S.; Zheng X.; Tan K.; Waksman G.; Sadler J.E.
CORPORATE SOURCE: J.E. Sadler, Howard Hughes Medical Institute, Department of Medicine, Washington University Sch. Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, United States. esadler@im.wustl.edu
SOURCE: Journal of Molecular Biology, (17 Sep 1999) 292/2 (361-373).
Refs: 45
ISSN: 0022-2836 CODEN: JMOBAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Enteropeptidase is a membrane-bound serine protease that initiates the activation of pancreatic **hydrolases** by cleaving and activating trypsinogen. The enzyme is remarkably specific and cleaves after lysine residues of peptidyl substrates that resemble trypsinogen activation peptides such as Val-(Asp)4-Lys. To characterize the determinants of substrate specificity, we solved the crystal structure of the bovine enteropeptidase catalytic domain to 2.3 .ANG. resolution in complex with the inhibitor Val-(Asp)4-Lys-chloromethane. The catalytic mechanism and contacts with lysine at substrate position P1 are conserved with other

trypsin-like serine proteases. However, the **aspartyl** residues at positions P2-P4 of the inhibitor interact with the enzyme surface mainly through salt bridges with the N(.zeta.) atom of Lys99. Mutation of Lys99 to Ala, or acetylation with acetic anhydride, specifically prevented the cleavage of trypsinogen or Gly-(Asp)4Lys-.**beta**.-naphthylamide and reduced the rate of inhibition by Val-(Asp)4Lys-chloromethane 22 to 90-fold. For these reactions, Lys99 was calculated to account for 1.8 to 2.5 kcal mol-1 of the free energy of transition state binding. Thus, a unique basic exosite on the enteropeptidase surface has evolved to facilitate the cleavage of its physiological substrate, trypsinogen.

L8 ANSWER 4 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1999:128226 BIOSIS
DOCUMENT NUMBER: PREV199900128226
TITLE: **Aspartyl** (Asparaginyl) **beta** **hydroxylase** (AAH) expression marks invasiveness of primary malignant CNS neoplasms.
AUTHOR(S): de La Monte, S. M.; Gemelli, B.; Brown, N. V.; Wands, J. R.
CORPORATE SOURCE: Massachusetts Gen. Hosp., Harvard Med. Sch., Boston, MA USA
SOURCE: Modern Pathology, (Jan., 1999) Vol. 12, No. 1, pp. 170A.
Meeting Info.: Annual Meeting of the United States and Canadian Academy of Pathology San Francisco, California, USA March 20-26, 1999
ISSN: 0893-3952.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 5 OF 56 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999358403 MEDLINE
DOCUMENT NUMBER: 99358403
TITLE: Development of a carbon dioxide-capture assay in microtiter plate for **aspartyl-beta** **-hydroxylase**.
AUTHOR: Zhang J H; Qi R C; Chen T; Chung T D; Stern A M; Hollis G F; Copeland R A; Oldenburg K R
CORPORATE SOURCE: DuPont Pharmaceuticals Research Laboratories, DuPont Pharmaceuticals Company, Wilmington, Delaware 19880-0400, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1999 Jul 1) 271 (2) 137-42.
Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911

ENTRY WEEK: 19991103

AB CO2-capture methods have been used for assaying many decarboxylating enzymes including hydroxylation-coupled decarboxylation reactions. The traditional CO2-capture method involves performing the reaction in capped tubes and radiometric measurement of trapped 14CO2 by scintillation counting. In this report, a 14CO2-capture method in a 96-well microtiter plate format has been developed and a phosphor imaging system has been employed for sample measurement. The new assay method has been used successfully to assay **aspartyl-beta-hydroxylase** activity in microtiter plate format. The results obtained here compare favorably with those obtained from the traditional tube method. The method is sensitive, suitable for high throughput, and generally applicable to many CO2-releasing enzyme assays.

L8 ANSWER 6 OF 56 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 1998434529 MEDLINE

DOCUMENT NUMBER: 98434529

TITLE: Glycosylasparaginase-catalyzed synthesis and hydrolysis of beta-aspartyl peptides.

AUTHOR: Noronkoski T; Stoineva I B; Ivanov I P; Petkov D D; Mononen I

CORPORATE SOURCE: Department of Clinical Chemistry, Kuopio University Hospital, P.O. Box 1777, FIN-70211 Kuopio, Finland.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Oct 9) 273 (41) 26295-7.
Journal code: HIV. ISSN: 0021-9258.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199901

ENTRY WEEK: 19990104

AB **beta-Aspartyl** di- and tripeptides are common constituents of mammalian metabolism, but their formation and catabolism are not fully understood. In this study we provide evidence that glycosylasparaginase (aspartylglucosaminidase), an N-terminal nucleophile **hydrolase** involved in the hydrolysis of the N-glycosidic bond in glycoproteins, catalyzes the hydrolysis of **beta-aspartyl** peptides to form L-aspartic acid and amino acids or peptides. The enzyme also effectively catalyzes the synthesis of **beta-aspartyl** peptides by transferring the **beta-aspartyl** moiety from other **beta-aspartyl** peptides or **beta**-aspartylglycosylamine to a variety of amino acids and peptides. Furthermore, the enzyme can use L-asparagine as the **beta-aspartyl** donor in the formation of **beta-aspartyl** peptides. The data show that synthesis and degradation of **beta-**

aspartyl peptides are new, significant functions of glycosylasparaginase and suggest that the enzyme could have an important role in the metabolism of **beta-aspartyl** peptides.

L8 ANSWER 7 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:790020 SCISEARCH
THE GENUINE ARTICLE: 125VQ
TITLE: The **aspartyl** (asparaginyl) **beta-hydroxylase** gene found overexpressed in human cholangiocarcinomas induces malignant transformation
AUTHOR: Ince N (Reprint); Wands J R
CORPORATE SOURCE: HARVARD UNIV, SCH MED, MGH E, CTR CANC, MOL HEPATOL LAB, CHARLESTOWN, MA 02129
COUNTRY OF AUTHOR: USA
SOURCE: HEPATOLOGY, (OCT 1998) Vol. 28, No. 4, Part 2, Supp. [S], pp. 1011-1011.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.
ISSN: 0270-9139.
DOCUMENT TYPE: Conférence; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 0

L8 ANSWER 8 OF 56 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998422342 MEDLINE
DOCUMENT NUMBER: 98422342
TITLE: The five cysteine residues located in the active site region of bovine **aspartyl** (asparaginyl) **beta-hydroxylase** are not essential for catalysis.
AUTHOR: McGinnis K; Ku G M; Fu J; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Research Laboratories, West Point, PA 19486, USA.
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1998 Sep 8) 1387 (1-2) 454-6.
Journal code: A0W. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104
AB In previous chemical modification studies on bovine **aspartyl** (asparaginyl) **beta-hydroxylase**, cysteines were implicated as critical catalytic residues. Using site-directed mutagenesis, the five cysteine residues located in a highly

conserved region of the enzyme identified as the active site were individually mutated to alanine. Substitutions at cysteine 637, 644, 656, 681, and 696 resulted in active mutant enzymes indicating that these residues are not required for catalysis.

L8 ANSWER 9 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:525967 BIOSIS
DOCUMENT NUMBER: PREV199800525967
TITLE: The **aspartyl** (asparaginyl) **beta-hydroxylase** gene found overexpressed in human cholangiocarcinomas induces malignant transformation.
AUTHOR(S): Ince, N.; Wands, J. R.
CORPORATE SOURCE: Molecular Hepatol. Lab., MGH East, Cancer Cent., Harvard Med. Sch., 149 13th St., Charlestown, MA 02129 USA
SOURCE: Hepatology, (Oct., 1998) Vol. 28, No. 4 PART 2, pp. 415A.
Meeting Info.: Biennial Scientific Meeting of the International Association for the Study of the Liver and the 49th Annual Meeting and Postgraduate Courses of the American Association for the Study of Liver Diseases Chicago, Illinois, USA November 4-10, 1998 International Association for the Study of the Liver . ISSN: 0270-9139.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 10 OF 56 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998291235 MEDLINE
DOCUMENT NUMBER: 98291235
TITLE: Aspartylglucosaminuria in a Canadian family.
AUTHOR: Gordon B A; Rupar C A; Rip J W; Haust M D; Coulter-Mackie M B; Scott E; Hinton G G
CORPORATE SOURCE: Department of Paediatrics, University of Western Ontario.
SOURCE: CLINICAL AND INVESTIGATIVE MEDICINE. MEDECINE CLINIQUE ET EXPERIMENTALE, (1998 Jun) 21 (3) 114-23. Journal code: DFG. ISSN: 0147-958X.
PUB. COUNTRY: Canada
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY WEEK: 19981005
AB Aspartylglucosaminuria (McKusick 208400) is a lysosomopathy associated with aspartylglucosaminidase (L-aspartamido-**beta**-N-acetylglucosamine amidohydrolase, EC 3.5.1.26) deficiency. It has been most frequently encountered in Finland, where the regional incidence may be as high as 1 in 3600 births. In North America it is

very rare, having been reported in only 8 patients. We encountered 4 patients with aspartylglucosaminuria in a Canadian family of 12 siblings. The 4 siblings affected--2 brothers and 2 sisters--were apparently normal at birth; however, their developmental milestones, particularly speech, were slow, and they acquired only a simple vocabulary. Throughout life, there was a progressive coarsening of facial features; 3 had inguinal hernia and recurrent diarrhea; all became severely retarded and by the 4th decade showed evident deterioration of both cognitive and motor skills; 2 exhibited cyclical behavioural changes. Three of the siblings have died, at 33, 39 and 44 years of age. Two died of bronchopneumonia and 1 of asphyxiation following aspiration. In the urine of all 4 siblings, and in the 1 liver examined, we found 2-acetamido-1-N-(4-L-**aspartyl**)-2-deoxy-**beta**-D-glucosamine (GlcNAc-Asn) and alpha-D-mannose-(1,6)-**beta**-D-mannose-(1,4)-2-acetamido-2-deoxy-**beta**-D-glucose-(1,4)-2-acetamido-1-N-(4-L-**aspartyl**)-2-deoxy-**beta** - D-glucosamine (Man2-GlcNAc2-Asn). Compared with the level of activity in controls, aspartylglucosaminidase activity was less than 2% in fibroblasts from 3 of the siblings, less than 0.5% in leukocytes from 1 sibling, and less than 1% in the liver of 1 sibling, whereas other acid **hydrolase** activities in these tissues were normal. Ultrastructural studies of skin showed that fibroblasts, endothelial cells and pericytes contained vacuoles with fine reticulo-floccular material. Glial and neuronal cells of the central nervous system showed similar inclusions as well as others composed of concentric or parallel membranous arrays intermingled with lipid droplets.

L8 ANSWER 11 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 7
ACCESSION NUMBER: 1997:279430 BIOSIS
DOCUMENT NUMBER: PREV199799578633
TITLE: Cellular overexpression of human asparaginyl/**aspartyl beta-hydroxylase** (**HAAH**) in gastric and colon cancers.
AUTHOR(S): Nishimaki, H. (1); Nishiyama, M.; Wands, J. R.; Tanaka, T. (1)
CORPORATE SOURCE: (1) Dep. Internal Med., Jikei Univ. Sch. Med., Tokyo Japan
SOURCE: Gastroenterology, (1997) Vol. 112, No. 4 SUPPL., pp. A628.
Meeting Info.: Digestive Disease Week and the 97th Annual Meeting of the American Gastroenterological Association Washington, D.C., USA May 11-14, 1997
ISSN: 0016-5085.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L8 ANSWER 12 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1997:237123 BIOSIS

DOCUMENT NUMBER: PREV199799536326
TITLE: Human aspartyl 3-hydroxylase: Recombinant expression and domain structure of an enzyme critical for extracellular proteins.
AUTHOR(S): Slowinska, Bozena (1); Zagulska, Sylwia (1); Frey, Jurgen; Chang, Patricia I.; Hananske-Abel, Hartmut M. (1)
CORPORATE SOURCE: (1) Dep. Pediatr., N.Y. Hosp.-Cornell Med. Cent., New York, NY USA
SOURCE: Pediatric Research, (1997) Vol. 41, No. 4 PART 2, pp. 107A.
Meeting Info.: Meeting of the American Pediatric Society and the Society for Pediatric Research Washington, D.C., USA May 2-6, 1997
ISSN: 0031-3998.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L8 ANSWER 13 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1997:230634 BIOSIS
DOCUMENT NUMBER: PREV199799529837
TITLE: Detection beta-aspartyl(asparaginyl) hydroxylation in Notch.
AUTHOR(S): Jia, S. (1); Ma, J.; Stern, A. M.; Corman, J.; Blom, K.; Weinmaster, G.; Friedman, P. A.
CORPORATE SOURCE: (1) Dupont Merck Res. Lab., Wilmington, DE 19880 USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (1997) Vol. 38, No. 0, pp. 64.
Meeting Info.: Eighty-eighth Annual Meeting of the American Association for Cancer Research San Diego, California, USA April 12-16, 1997
ISSN: 0197-016X.
DOCUMENT TYPE: Conference; Abstract
LANGUAGE: English

L8 ANSWER 14 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 97:876858 SCISEARCH
THE GENUINE ARTICLE: YH077
TITLE: Immunological approach to hepatocellular carcinoma
AUTHOR: Wands J R (Reprint); Lavaissiere L; Moradpour D; delaMonte S; Mohr L; Nicolau C; Tanaka S
CORPORATE SOURCE: MASSACHUSETTS GEN HOSP, CTR CANC, MOL HEPATOL LAB, BLDG 149 13TH ST, 7TH FLOOR, CHARLESTOWN, MA 02129 (Reprint); HARVARD UNIV, SCH MED, CHARLESTOWN, MA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF VIRAL HEPATITIS, (NOV-DEC 1997) Vol. 4, Supp. [2], pp. 60-74.
Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD,

OXFORD, OXON, ENGLAND OX2 0EL.
ISSN: 1352-0504.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: CLIN
LANGUAGE: English
REFERENCE COUNT: 72

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A library of monoclonal antibodies (MoAbs) has been produced against a human hepatocellular carcinoma (HCC) cell line designated FOCUS in order to study the antigenic properties of transformed hepatocytes. Several monoclonal antibodies (MoAbs) were initially selected for study since they bound to antigens which were overexpressed in HCC tissues compared with the adjacent uninvolved normal liver counterpart: in addition, these MoAbs revealed low level antigen expression on other normal human tissues, Subsequently, HCC cell lines were metabolically labelled and the antigens further characterized by immunoprecipitation and Western blot analysis, If the MoAb recognized a primary linear epitope on a protein, cloning was performed using a lambda GT11 cDNA expression library prepared from the FOCUS HCC cell line, These studies characterized the HCC associated antigen(si at the molecular level, This review illustrates the value of such an experimental approach to search for and identify HCC associated antigens and emphasizes the biological properties of novel proteins may be defined and characterized by these techniques. More important, our investigations have described unique proteins that may not only be important in the pathogenesis of HCC IT but also demonstrates how such antigen-antibody systems may be used to develop strategies for immuno-targetting and gene therapy of HCC.

L8 ANSWER 15 OF 56 MEDLINE DUPLICATE 8

ACCESSION NUMBER: 96264773 MEDLINE
DOCUMENT NUMBER: 96264773

TITLE: Site-directed mutagenesis of residues in a conserved region of bovine **aspartyl** (asparaginyl) **beta-hydroxylase**: evidence that histidine 675 has a role in binding Fe2+.

AUTHOR: McGinnis K; Ku G M; VanDusen W J; Fu J; Garsky V; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Research Laboratories, West Point, Pennsylvania 19486, USA.

SOURCE: BIOCHEMISTRY, (1996 Apr 2) 35 (13) 3957-62.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610

AB The roles in catalysis of several residues in bovine

aspartyl (asparaginyl) **beta-hydroxylase**
that are located in a region of homology among alpha-ketoglutarate-dependent dioxygenases were investigated using site-directed mutagenesis. Previous studies have shown that when histidine 675, an invariant residue located in this highly conserved region, was mutated to an alanine residue, no enzymatic activity was detected. A more extensive site-directed mutagenesis study at position 675 has been undertaken to define the catalytic role of this essential residue. The partial **hydroxylase** activity observed with some amino acid replacements for histidine 675 correlates with the potential to coordinate metals and not with size, charge, or hydrophobic character. Furthermore, the increase in Km for Fe2+ observed with the H675D and H675E mutant enzymes can account for their partial activities relative to wild type. No significant changes in the Km for alpha-ketoglutarate (at saturating Fe2+) or Vmax were observed for these mutants. These results support the conclusion that histidine 675 is specifically involved in Fe2+ coordination. Further site-directed mutagenesis of other highly conserved residues in the vicinity of position 675 demonstrates the importance of this region of homology in catalysis for Asp (Asn) **beta-hydroxylase** and, by analogy, other alpha-ketoglutarate-dependent dioxygenases.

L8 ANSWER 16 OF 56 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 96420598 MEDLINE
DOCUMENT NUMBER: 96420598
TITLE: Overexpression of human **aspartyl** (asparaginyl)**beta-hydroxylase** in hepatocellular carcinoma and cholangiocarcinoma.
AUTHOR: Lavaissiere L; Jia S; Nishiyama M; de la Monte S; Stern A M; Wands J R; Friedman P A
CORPORATE SOURCE: Molecular Hepatology Laboratory, Massachusetts General Hospital Cancer Center, Charlestown 02129, USA.
CONTRACT NUMBER: CA-35711 (NCI)
AA-08169 (NIAAA)
AA-02666 (NIAAA)
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1996 Sep 15) 98 (6) 1313-23.
Journal code: HS7. ISSN: 0021-9738.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-S83325
ENTRY MONTH: 199701
AB To characterize genes that become upregulated with malignant transformation of human hepatocytes, a library of monoclonal

antibodies was produced against the FOCUS hepatocellular carcinoma cell line. Antibody FB-50 reacted with an antigen that was highly expressed in 4 of 10 primary hepatocellular carcinomas, in all 20 cholangiocarcinomas we studied, and in a variety of transformed cell lines. This antigen was also highly expressed in neoplastic epithelial cells of breast and colon carcinomas in contrast to its low level of expression in normal hepatocytes and in non-neoplastic epithelial cells. Among the normal adult tissues studied, high levels were observed only in proliferating trophoblastic cells of the placenta and in adrenal glands. A 636-bp partial cDNA, isolated from a gamma GT11 expression library generated with HepG2 human hepatoblastoma cells, and a complete cDNA, generated by reverse transcriptase-PCR, identified the antigen as the human form of **aspartyl**(asparaginyl)**beta-hydroxylase**.
This enzyme catalyzes posttranslational hydroxylation of **beta** carbons of specific **aspartyl** and asparaginyl residues in EGF-like domains of certain proteins. Analyses of extracts prepared from several human tumor cell lines compared to their normal tissue counterparts indicate that the increase in **hydroxylase**, approximately 10-fold, is controlled at the level of transcription and the protein is expressed in an enzymatically active form. In similar analyses, comparing hepatocellular carcinomas to adjacent uninvolved liver from five patients, enzymatic activity was much higher in the tumor tissue from the four patients whose immunoblots revealed increased **hydroxylase** protein in the malignant tissue. EGF repeats in the extracellular domain of Notch or its homologs contain the consensus sequence for hydroxylation. Deletion mutants lacking this domain are gain-of-function mutants, suggesting that the domain modulates signal transduction by the cytoplasmic domain. While the function imparted by **beta** hydroxylation is unknown, our studies raise the possibility that **beta** hydroxylation is regulated in proteins like the mammalian Notch homologs, whose cytoplasmic domains have been shown to be oncogenic.

L8 ANSWER 17 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1996:140773 BIOSIS
DOCUMENT NUMBER: PREV199698712908
TITLE: Cloning of JUNCTIN, a major calsequestrin (CSQ) binding protein of junctional sarcoplasmic reticulum (SR.
AUTHOR(S): Zhang, Lin (1); Jorgensen, Annelise; Jones, Larry
CORPORATE SOURCE: (1) Krannert Inst. Cardiol., Indiana Univ. Sch. Med., Indianapolis, IN 46202 USA
SOURCE: Biophysical Journal, (1996) Vol. 70, No. 2 PART 2, pp. A163.
Meeting Info.: 40th Annual Meeting of the Biophysical Society Baltimore, Maryland, USA February 17-21, 1996
ISSN: 0006-3495.

DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 18 OF 56 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96107245 MEDLINE
DOCUMENT NUMBER: 96107245
TITLE: Purification, primary structure, and immunological characterization of the 26-kDa calsequestrin binding protein (junctin) from cardiac junctional sarcoplasmic reticulum.
AUTHOR: Jones L R; Zhang L; Sanborn K; Jorgensen A O; Kelley J
CORPORATE SOURCE: Department of Medicine, Indiana University School of Medicine, Indianapolis 46202, USA.
CONTRACT NUMBER: HL28556 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 22) 270 (51) 30787-96.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-U38414
ENTRY MONTH: 199604

AB Previously we identified a protein of apparent M(r) = 26,000 as the major calsequestrin binding protein in junctional sarcoplasmic reticulum vesicles isolated from cardiac and skeletal muscle (Mitchell, R. D., Simmerman, H. K. B., and Jones, L. R. (1988) J. Biol. Chem. 263, 1376-1381). Here we describe the purification and primary structure of the 26-kDa calsequestrin binding protein. The protein was purified 164-fold from cardiac microsomes and shown by immunoblotting to be highly enriched in junctional membrane subfractions. It ran as a closely spaced doublet on SDS-polyacrylamide gel electrophoresis and bound 125I-calsequestrin intensely. Cloning of the cDNA predicted a protein of 210 amino acids containing a single transmembrane domain. The protein has a short N-terminal region located in the cytoplasm, and the bulk of the molecule, which is highly charged and basic, projects into the sarcoplasmic reticulum lumen. Significant homologies were found with triadin and **aspartyl beta-hydroxylase**, suggesting that all three proteins are members of a family of single membrane-spanning endoplasmic reticulum proteins. Immunocytochemical labeling localized the 26-kDa protein to junctional sarcoplasmic reticulum in cardiac and skeletal muscle. The same gene product was expressed in these two tissues. The calsequestrin binding activity of the 26-kDa protein combined with its codistribution with calsequestrin and ryanodine receptors strongly suggests that the protein plays an important role in the organization and/or function of the Ca2+ release complex. Because the 26-kDa calsequestrin

binding protein is an integral component of the junctional sarcoplasmic reticulum membrane in cardiac and skeletal muscle, we have named it Junctin.

L8 ANSWER 19 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1995:286006 BIOSIS
DOCUMENT NUMBER: PREV199598300306
TITLE: Human **aspartyl**(asparaginyl) **beta-hydroxylase** is highly active in human lung carcinoma A459 cells and human hepatocellular carcinoma HEP G2 cells.
AUTHOR(S): Iia, S.; Kuo, A.; Stern, A. M.; Friedman, P. A.
CORPORATE SOURCE: Dep. Appl. Biotechnol., DuPont Merck Pharmaceutical Co., 500 S. Ridgeway Ave., Glenolden, PA 19036 USA
SOURCE: FASEB Journal, (1995) Vol. 9, No. 6, pp. A1263. Meeting Info.: Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA May 21-25, 1995
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 20 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 95:325610 SCISEARCH
THE GENUINE ARTICLE: QV274
TITLE: HUMAN **ASPARTYL**(ASPARAGINYL) **BETA** -**HYDROXYLASE** IS HIGHLY-ACTIVE IN HUMAN LUNG-CARCINOMA A459 CELLS AND HUMAN HEPATOCELLULAR-CARCINOMA HEP G2 CELLS
AUTHOR: JIA S (Reprint); KUO A; STERN A M; FRIEDMAN P A
CORPORATE SOURCE: DUPONT MERCK PHARMACEUT CO, DEPT APPL BIOTECH, GLENOLDEN, PA, 19036
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (24 APR 1995) Vol. 9, No. 6, pp. A1263.
ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: No References

L8 ANSWER 21 OF 56 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 94316668 MEDLINE
DOCUMENT NUMBER: 94316668
TITLE: A fully active catalytic domain of bovine **aspartyl** (asparaginyl) **beta-hydroxylase** expressed in Escherichia coli: characterization and evidence for the identification of an active-site region in vertebrate

alpha-ketoglutarate-dependent dioxygenases.
AUTHOR: Jia S; McGinnis K; VanDusen W J; Burke C J; Kuo A; Griffin P R; Sardana M K; Elliston K O; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Research Laboratories, West Point, PA 19486..
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Jul 19) 91 (15) 7227-31.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199410
AB The alpha-ketoglutarate-dependent dioxygenase **aspartyl** (asparaginyl) **beta-hydroxylase** (EC 1.14.11.16) specifically hydroxylates one aspartic or asparagine residue in certain epidermal growth factor-like domains of a number of proteins. The expression in Escherichia coli, purification, characterization of a fully active catalytic domain, and evidence for the identification of an active-site region of this enzyme are described. Sequence alignment analyses among the vertebrate alpha-ketoglutarate-dependent dioxygenases and chemical modification studies were undertaken aimed at locating specific regions of 52-kDa recombinant **aspartyl** (asparaginyl) **beta-hydroxylase** involved in substrate binding and/or catalysis. Based upon these studies, an alignment of the C-terminal regions of prolyl and lysyl **hydroxylase** and of **aspartyl** (asparaginyl) **beta-hydroxylase** is proposed. When histidine-675, an invariant residue located in a region of homology within this alignment, was mutated to an alanine residue in **aspartyl** (asparaginyl) **beta-hydroxylase** (H675A), no enzymatic activity was detected. Chemical modification studies show that the wild-type protein is protected from iodo[14C]acetamide labeling by Fe2+/alpha-ketoglutarate whereas the H675A mutant protein is not, suggesting that this mutant does not bind Fe2+/alpha-ketoglutarate.

L8 ANSWER 22 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 12
ACCESSION NUMBER: 1994:334471 BIOSIS
DOCUMENT NUMBER: PREV199497347471
TITLE: Identification of active site residues in bovine **aspartyl** (asparaginyl) **beta-hydroxylase** by site-directed mutagenesis.
AUTHOR(S): McGinnis, K.; Vandusen, W. J.; Ku, G.; Stern, A. M.; Friedman, P. A.
CORPORATE SOURCE: Merck Res. Lab., West Point, PA 19486 USA
SOURCE: FASEB Journal, (1994) Vol. 8, No. 7, pp. A1369.
Meeting Info.: 85th Annual Meeting of the American

Society for Biochemistry and Molecular Biology
Washington, D.C., USA May 21-25, 1994
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 23 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 94:507146 SCISEARCH
THE GENUINE ARTICLE: PC036
TITLE: 2-OXOGLUTARATE-DEPENDENT DIOXYGENASE AND RELATED ENZYMES - BIOCHEMICAL-CHARACTERIZATION
AUTHOR: DECAROLIS E (Reprint); DELUCA V
CORPORATE SOURCE: UNIV MONTREAL, INST RECH BIOL VEGETALE, DEPT BIOL SCI, 4101 RUE SHERBROOKE, MONTREAL H1X 2B2, PQ, CANADA (Reprint)
COUNTRY OF AUTHOR: CANADA
SOURCE: PHYTOCHEMISTRY, (JUL 1994) Vol. 36, No. 5, pp. 1093-1107.
ISSN: 0031-9422.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 120
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hydroxylation reactions are catalysed by a few major subclasses of enzymes which are ubiquitously distributed in nature. Dioxygenases generally occur as soluble enzymes where they catalyse a diversity of oxygenation reactions in a large number of metabolic pathways in animals, plants and micro-organisms. This review discusses recent advances in the biochemistry and molecular biology of dioxygenases occurring in different biological systems.

L8 ANSWER 24 OF 56 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 95121937 MEDLINE
DOCUMENT NUMBER: 95121937
TITLE: Cloning and characterization of the human gene encoding **aspartyl beta-hydroxylase**.
AUTHOR: Korioth F; Gieffers C; Frey J
CORPORATE SOURCE: Universitat Bielefeld, Fakultat fur Chemie, Germany.
SOURCE: GENE, (1994 Dec 15) 150 (2) 395-9.
Journal code: FOP. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U03109
ENTRY MONTH: 199504
AB Sequence information for **aspartyl beta-**

hydroxylase (AspH), which specifically hydroxylates one Asp or Asn residue in certain epidermal growth factor (EGF)-like domains of a number of proteins, is so far only described for bovine species. We have isolated a 4.3-kb cDNA encoding the human AspH (hAspH) by immunoscreening of a human osteosarcoma (MG63) cDNA library in lambda ZAP with an antiserum raised against membrane fractions of these cells. Northern blot analyses revealed two transcripts with lengths of 2.6 and 4.3 kb. The deduced amino acid (aa) sequence of this cDNA encodes a protein of 757 aa (85 kDa). Comparison with the deduced bovine AspH (bAspH) aa sequence showed striking differences in the N-terminal portion of this protein. In vitro transcription and translation in the presence of canine pancreas microsomes yielded a 56-kDa protein. Western blot analyses of membrane fractions from MG63 cells with AspH-specific antibodies revealed a protein of the same M(r). These results suggest a posttranslational cleavage of the catalytic C terminus in the lumen of the endoplasmic reticulum.

L8 ANSWER 25 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1995:3070 BIOSIS
DOCUMENT NUMBER: PREV199598017370
TITLE: Molecular characterization and cloning of FB-50 antigen highly expressed in human hepatocellular carcinoma.
AUTHOR(S): Lavaissiere, L. (1); Wands, J. R.; Takahashi, H.
CORPORATE SOURCE: (1) Mol. Hepatol. Lab., MGH Cancer Cent., Charlestown, MA USA
SOURCE: Hepatology, (1994) Vol. 20, No. 4 PART 2, pp. 275A. Meeting Info.: 45th Annual Meeting of the American Association for the Study of Liver Diseases Chicago, Illinois, USA November 11-15, 1994
ISSN: 0270-9139.
DOCUMENT TYPE: Conference
LANGUAGE: English

L8 ANSWER 26 OF 56 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 93:325200 SCISEARCH
THE GENUINE ARTICLE: LB745
TITLE: GAMMA-BUTYROBETAINE HYDROXYLASE STRUCTURAL CHARACTERIZATION OF THE PSEUDOMONAS ENZYME
AUTHOR: RUETSCHI U (Reprint); NORDIN I; ODELHOG B; JORNVALL H; LINDSTEDT S
CORPORATE SOURCE: GOTHENBURG UNIV, SAHLGRENS HOSP, DEPT CLIN CHEM, S-41345 GOTHENBURG, SWEDEN (Reprint); KAROLINSKA INST, DEPT CHEM 1, S-10401 STOCKHOLM 60, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (01 MAY 1993) Vol. 213, No. 3, pp. 1075-1080.
ISSN: 0014-2956.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Gamma-Butyrobetaine hydroxylase is a 2-oxoglutarate-dependent dioxygenase that catalyzes the hydroxylation of y-butyrobetaine to carnitine, the last step in the biosynthesis of carnitine from lysine. The primary structure of the enzyme from Pseudomonas sp. AK1 has been determined. Sequence analysis of the intact protein and of peptides from essentially three different digests established the presence of a peptide chain containing 383 residues, and an N-terminal truncated form of 382 residues. The two chains have molecular masses of 43 321 Da and 43 207 Da, respectively, and are identical except for the presence or absence of an N-terminal asparagine residue; the shorter form starts with an alanine residue. In preparations of the dimeric protein, the two chains occur in an approximate ratio of 1 : 1. There are nine cysteine residues and 13 histidine residues, i. e. amino acids which have been postulated as ligands for iron binding. In spite of functional similarities, there appears to be no clear sequence similarities with any of the other mammalian 2-oxoglutarate-dependent dioxygenases so far characterized.

L8 ANSWER 27 OF 56 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 92332546 MEDLINE
DOCUMENT NUMBER: 92332546
TITLE: cDNA cloning and expression of bovine **aspartyl** (asparaginyl) **beta-hydroxylase**.
AUTHOR: Jia S; VanDusen W J; Diehl R E; Kohl N E; Dixon R A; Elliston K O; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Jul 15) 267 (20) 14322-7.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-M91213; GENBANK-L08495; GENBANK-X62322; GENBANK-M63383; GENBANK-M86615; GENBANK-M86616; GENBANK-M86617; GENBANK-M86618; GENBANK-M86619; GENBANK-M86620
ENTRY MONTH: 199210

AB **Aspartyl** (asparaginyl) **beta-hydroxylase** which specifically hydroxylates 1 Asp or Asn residue in certain epidermal growth factor-like domains of a number of proteins, has

been previously purified to apparent homogeneity from detergent-solubilized bovine liver microsomes (Wang, Q., VanDusen, W. J., Petroski, C. J., Garsky, V. M., Stern, A. M., and Friedman, P. A. (1991) J. Biol. Chem. 266, 14004-14010). Three oligonucleotides, corresponding to three amino acid sequences of the purified **hydroxylase**, were used to screen bovine cDNA libraries. Several overlapping positive cDNA clones containing a full length open reading frame of 754 amino acids encoding a 85-kDa protein were isolated, and a cDNA, containing the full length open reading frame, was constructed from two of these clones. The resulting clone was then transcribed and translated in vitro to produce recombinant protein which possessed Asp **beta-hydroxylase** activity. These results constitute proof that the protein purified from bovine liver is an Asp **beta-hydroxylase**. Comparisons of deduced amino acid sequences of two other alpha-ketoglutarate-dependent dioxygenases, prolyl-4-**hydroxylase** and lysyl **hydroxylase**, with that of Asp **beta-hydroxylase** showed no significant homologies. Indeed, Asp **beta-hydroxylase** appears to be unique as no striking homology was found with known protein sequences. Furthermore, structural predictions derived from the deduced amino acid sequence are in accord with earlier Stokes' radius and sedimentation coefficient determinations of the enzyme, suggesting that the enzyme contains a relatively compact carboxyl-terminal catalytic domain and an extended amino terminus. This amino-terminal region has a potential transmembrane type II signal-anchor domain that could direct the catalytic domain into the lumen of the endoplasmic reticulum.

L8 ANSWER 28 OF 56 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 92202239 MEDLINE
DOCUMENT NUMBER: 92202239
TITLE: Substrate specificity and reaction mechanism of human glycoasparaginase. The N-glycosidic linkage of various glycoasparagines is cleaved through a reaction mechanism similar to L-asparaginase.
AUTHOR: Kaartinen V; Mononen T; Laatikainen R; Mononen I
CORPORATE SOURCE: Department of Clinical Chemistry, Kuopio University Central Hospital, Finland.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Apr 5) 267 (10) 6855-8.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199207
AB Human glycoasparaginase (N4-(**beta**-N-acetyl-D-glucosaminyl)-L-asparaginase, EC 3.5.1.26) hydrolyzes a series of compounds that

contain L-asparagine residue with free alpha-amino and alpha-carboxyl groups. Substrates include high mannose and complex type glycoasparagines as well as those that lack the di-N-acetylchitobiose moiety, L-aspartic acid **beta**-methyl ester and L-aspartic acid **beta**-hydroxamate. The enzyme is inactive toward L-asparagine and L-glutamine and glycoasparagines containing substituted alpha-amino and/or alpha-carboxyl groups. In the presence of the acyl acceptor hydroxylamine, glycoasparaginase catalyzes the synthesis of L-aspartic acid **beta** -hydroxamate from **aspartyl**-glucosamine, L-aspartic acid **beta**-methyl ester, and L-aspartic acid. 13C NMR studies using 18O-labeled L-aspartic acid demonstrate that glycoasparaginase catalyzes an oxygen exchange between water and the carboxyl group at C-4 of L-aspartic acid. These results indicate that glycoasparaginase reacts as an exo-**hydrolase** toward the L-asparagine moiety of the substrates and the free alpha-amino and alpha-carboxyl groups are required for the enzyme reaction. The results are consistent with an L-asparaginase-like reaction pathway which involves a **beta-aspartyl** enzyme intermediate. Since glycoasparaginase is active toward a series of structurally different glycoasparagines, we suggest the revised systematic name of N4-(**beta**-glycosyl)-L-asparaginase for the enzyme.

L8 ANSWER 29 OF 56 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 93080563 MEDLINE
DOCUMENT NUMBER: 93080563
TITLE: Invertebrate **aspartyl**/asparaginyl **beta-hydroxylase**: potential modification of endogenous epidermal growth factor-like modules.
AUTHOR: Monkovic D D; VanDusen W J; Petroski C J; Garsky V M; Sardana M K; Zavodszky P; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1992 Nov 30) 189 (1) 233-41.
Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199303
AB An invertebrate alpha-ketoglutarate-dependent **aspartyl** /asparaginyl **beta-hydroxylase**, which posttranslationally hydroxylates specific **aspartyl** or asparaginyl residues within epidermal growth factor-like modules, was identified, partially purified and characterized. Preparations derived from two insect cell lines catalyzed the hydroxylation of

the expected asparaginyl residue within a synthetic epidermal growth factor-like module. This activity was found to be similar to that of the purified mammalian **aspartyl**/asparaginyl **beta -hydroxylase** with respect to cofactor requirements, stereochemistry and substrate sequence specificity. Furthermore, recombinant human C1r, expressed in an insect cell-derived baculovirus expression system, was also found to be hydroxylated at the expected asparaginyl residue. Thus, these results establish the potential for invertebrate **aspartyl**/asparaginyl hydroxylation. Since several invertebrate proteins known to be required for proper embryonic development contain a putative consensus sequence that may be required for hydroxylation, the studies presented here provide the basis for further investigations concerned with identifying hydroxylated invertebrate proteins and determining their physiologic function.

L8 ANSWER 30 OF 56 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 91310689 MEDLINE
DOCUMENT NUMBER: 91310689
TITLE: Bovine liver **aspartyl beta-hydroxylase**. Purification and characterization.
AUTHOR: Wang Q P; VanDusen W J; Petroski C J; Garsky V M; Stern A M; Friedman P A
CORPORATE SOURCE: Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1991 Jul 25) 266 (21) 14004-10.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199110
AB The alpha-ketoglutarate-dependent dioxygenase, L-asp(L-Asn)-beta-hydroxylase which posttranslationally hydroxylates specific aspartic acid (asparagine) residues within epidermal growth factor-like domains was purified from bovine liver and characterized. A 52-kDa and a 56-kDa species of this enzyme, which accounted for 60 and 30% of the total enzymatic activity, respectively, were purified to apparent homogeneity. Amino-terminal sequence analyses and immunoblots utilizing antisera raised to the intact 52-kDa species as well as to two complementary fragments of this species demonstrated that the 52- and 56-kDa species differ by a 22-amino acid amino-terminal extension. The remaining 10% of the purified enzymatic activity could be accounted for by the presence of immunologically related higher molecular mass forms (56-90 kDa) of L-Asp(L-Asn)-beta-hydroxylase. Strong evidence was obtained from the results of immunoextraction studies that L-Asp(L-Asn)-beta-

hydroxylase can be identified with the purified proteins. Kinetic and physical studies suggest that L-Asp(L-Asn)-beta-hydroxylase exists as a monomer with a compact catalytic domain and an extended protease-sensitive amino terminus whose function remains to be determined. Since the purified L-Asp(L-Asn)-beta-hydroxylase hydroxylated both L-Asp- and L-Asn-containing substrates, it is possible that a single enzyme is responsibld occurred only at the aspartate corresponding to the position seen in vivo. Furthermore, the extent to which either peptide (factor IX or X) was hydroxylated reflected the extent of hydroxylation observed for both human plasma factors IX and X.

L8 ANSWER 33 OF 56 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 89264473 MEDLINE
DOCUMENT NUMBER: 89264473
TITLE: **Aspartyl beta-hydroxylase** : in vitro hydroxylation of a synthetic peptide based on the structure of the first growth factor-like domain of human factor IX.
AUTHOR: Gronke R S; VanDusen W J; Garsky V M; Jacobs J W; Sardana M K; Stern A M; Friedman P A
CORPORATE SOURCE: Department of Pharmacology, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1989 May) 86 (10) 3609-13.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198909
AB **beta**-Hydroxylation of aspartic acid is a post-translational modification that occurs in several vitamin K-dependent coagulation proteins. By use of a synthetic substrate comprised of the first epidermal growth factor-like domain in human factor IX and either mouse L-cell extracts or rat liver microsomes as the source of enzyme, in vitro **aspartyl beta** -hydroxylation was accomplished. **Aspartyl beta-hydroxylase** appears to require the same cofactors as known alpha-ketoglutarate-dependent dioxygenases. The hydroxylation reaction proceeds with the same stereospecificity and occurs only at the aspartate corresponding to the position seen in vivo. Further purification and characterization of this enzymatic activity should now be possible.

L8 ANSWER 34 OF 56 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 88295089 MEDLINE
DOCUMENT NUMBER: 88295089

TITLE: Protein-carboxyl methylation in adrenal medullary cells.
AUTHOR: Gagnon C; Veeraragavan K; Coulombe R
CORPORATE SOURCE: Urology Research Laboratory, Royal Victoria Hospital, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.
SOURCE: CELLULAR AND MOLECULAR NEUROBIOLOGY, (1988 Mar) 8 (1) 95-103. Ref: 44
Journal code: CPX. ISSN: 0272-4340.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198811

AB 1. The protein-carboxyl methylating system has been studied in adrenal medullary cells either using disrupted cell components or with intact cells. Whereas the enzyme protein-carboxyl methylase (PCM) is cytosolic, the majority of its substrates is on or within chromaffin granules. With intact granules, methylation of surface proteins results in solubilization of membrane proteins. 2. Membrane PCM substrates have been identified as two proteins with apparent molecular weights of 55,000 and 32,000. Among the substrates located inside the granules, the chromogranins are excellent substrates, while dopamine **beta-hydroxylase** is poorly methylated. 3. Under physiological conditions, stimulation of the splanchnic nerve results in an increase in adrenal medullary protein-methyl ester formation as well as in an augmented methanol production. With adrenal medullary cells in culture, carboxyl-methylated chromogranin A is detected in mature chromaffin granules between 3 and 6 hr after labeling. Methylated chromogranins are secreted concomitantly with catecholamines following cholinergic stimulation. 4. These data coupled with those of Chelsky et al. (J. Biol. Chem. 262:4303-4309, 1987) on lamin B suggest that PCM methylates residues other than D-**aspartyl** and L-isoaspartyl in proteins. They further suggest that methylation may occur on nascent peptide chains before they are injected into the rough endoplasmic reticulum.

L8 ANSWER 35 OF 56 MEDLINE
ACCESSION NUMBER: 89100781 MEDLINE
DOCUMENT NUMBER: 89100781
TITLE: Co-localization of N-acetyl-aspartyl-glutamate in central cholinergic, noradrenergic, and serotonergic neurons.
AUTHOR: Forloni G; Grzanna R; Blakely R D; Coyle J T
CORPORATE SOURCE: Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

CONTRACT NUMBER: NS 13584 (NINDS)
NS 18414 (NINDS)
SOURCE: SYNAPSE, (1987) 1 (5) 455-60.
Journal code: VFL. ISSN: 0887-4476.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198904

AB An immunohistochemical technique for simultaneously visualizing two different antigens has been used to investigate the presence of the acidic dipeptide, N-acetyl-**aspartyl**-glutamate (NAAG), in cholinergic, noradrenergic-adrenergic, and serotonergic neurons within CNS. The brain slices were processed sequentially with purified antisera against NAAG and then monoclonal antibody against choline acetyltransferase (ChAT), a marker for cholinergic neurons, or antiserum against dopamine-**beta-hydroxylase** (DBH), a marker of noradrenergic-adrenergic neurons, or antiserum against serotonin (5HT). Both antigens were revealed by the peroxidase reaction but with different chromogens, which are easily distinguishable. An intense double staining of NAAG-like immunoreactivity (NAAG-LI) and ChAT was observed in the motoneurons of the spinal cord as well as in the several motor components of cranial nerve nuclei including facial, ambiguus, and trigeminal nuclei. A partial colocalization of NAAG-LI and ChAT was evident in the perikarya of the basal forebrain cholinergic system, whereas cholinergic neurons of the medial septum exhibited only sporadic staining for NAAG-LI. A complete coexistence of NAAG-LI and DBH was observed in the locus coeruleus. Most of the other noradrenergic and adrenergic cell groups of the medulla region exhibited substantial co-localization with the exception of the A2 cell group, which was virtually devoid of NAAG-LI. In the dorsal raphe, only a low percentage of serotonergic neurons stained for NAAG-LI. The co-existence of NAAG-LI and serotonin was more evident in the neurons of the median raphe, although the majority of cells failed to show double staining.(ABSTRACT TRUNCATED AT 250 WORDS)

L8 ANSWER 36 OF 56 MEDLINE DUPLICATE 21

ACCESSION NUMBER: 85000565 MEDLINE
DOCUMENT NUMBER: 85000565
TITLE: Purification and partial characterization of aminopeptidase A from the serum of pregnant and non-pregnant women.
AUTHOR: Lalu K; Lampelo S; Nummelin-Kortelainen M; Vanha-Perttula T
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1984 Sep 25) 789 (3) 324-33.
Journal code: A0W. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198501
AB Aminopeptidase A (L-alpha-**aspartyl**(L-alpha-glutamyl)-peptide **hydrolase**, EC 3.4.11.7) was purified from human maternal and control sera using CM-cellulose chromatography, DEAE-Sephacel chromatography, Sephacryl S-300 gel filtration and hydroxyapatite chromatography. The purification coefficients were 3069 and 5210 and the yields 6.3 and 6.1% for the maternal and control serum, respectively. The purified enzymes appeared free from other serum aminopeptidases in polyacrylamide gel electrophoresis. The biochemical and physical characteristics of the enzymes from maternal and control sera were similar. A molecular weight of 260 000, an optimum at pH 6.75-7.25 and a fairly good stability of the enzymes at 4 and -18 degrees C were recorded. The alkaline earth metals (Ca2+, Ba2+, Sr2+) were the activators of alpha-L-glutamyl-**beta**-naphthylamide hydrolysis, while alpha-L-**aspartyl**-**beta**-naphthylamide hydrolysis was markedly potentiated with Ca2+ but not with Ba2+ at all. The most rapid hydrolysis was shown with GluNA (Km with Ba2+ 0.156 +/- 0.014 mM and 0.136 +/- 0.009 mM in maternal and control serum, respectively), while only minimal hydrolysis of some neutral and basic amino-acid-**beta**-naphthylamides were observed. The contribution of the placenta to the elevated aminopeptidase A levels in the pregnancy plasma could not be solved on the basis of the present observations.

L8 ANSWER 37 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1981:268866 BIOSIS
DOCUMENT NUMBER: BA72:53850
TITLE: REGIONAL DISTRIBUTION OF GLYCO ASPARAGINE STORAGE MATERIAL IN THE BRAIN IN ASPARTYL GLYCOSAMINURIA.
AUTHOR(S): MAURY C P J; HALTIA M; PALO J
CORPORATE SOURCE: DEP. MED. CHEM., UNIV. HELSINKI, SILTAVUORENPENGER 10 A, SF-00170 HELSINKI 17, FINL.
SOURCE: J NEUROL SCI, (1981) 50 (2), 291-298.
CODEN: JNSCAG. ISSN: 0022-510X.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The regional distribution of glycoasparagine storage material in the [human] brain was studied in aspartylglycosaminuria, a condition characterized by inherited deficiency of lysosomal N-aspartyl-.beta.-N-acetylglucosamine amidohydrolase. Gas-chromatographic measurements of the main accumulating glycoprotein-derived metabolite, N-acetylglucosaminyl-asparagine (GlcNAc-Asn), in 12 defined cerebral areas showed that GlcNAc-Asn is rather evenly distributed in the brain. The mean concentrations ranged from 0.454 mg/g wet tissue (corpus callosum)-0.610 mg/g

(pons). The GlcNAc-Asn concentrations tended to be higher in grey matter areas than in white matter areas. GlcNAc-Asn was identified in the isolated neuronal fraction, but not in the myelin fraction, by mass-fragmentographic techniques. EM reexamination of a brain biopsy specimen revealed, in addition to the abundant presence of storage lysosomes in the neuronal perikarya, numerous cytoplasmic inclusions in brain capillary endothelial cells and pericytes and in occasional macrophages. The glycoasparagine storage material apparently is not limited to expected cortical areas in aspartylglycosaminuria, but is distributed in a rather constant fashion in all cerebral grey and white matter areas studied.

L8 ANSWER 38 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 22
ACCESSION NUMBER: 1980:244821 BIOSIS
DOCUMENT NUMBER: BA70:37317
TITLE: EFFECTS OF PH TREATMENTS AND DEGLYCOSYLATION OF RABBIT IMMUNO GLOBULIN G ON THE BINDING OF COMPLEMENT C-1Q.
AUTHOR(S): WINKELHAKE J L; KUNICKI T J; ELCOMBE B M; ASTER R H
CORPORATE SOURCE: DEP. MICROBIOL., MED. COLL. WIS., MILWAUKEE, WIS., 53226, USA.
SOURCE: J BIOL CHEM, (1980) 255 (7), 2822-2828.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The abilities of deglycosylated or pH-denatured and renatured rabbit immunoglobulin (Ig)G antibodies to interact with antigen and the 1st component of complement, C1q, were studied to begin probing the environmental features of Ig domains required for the 1st step in activating the classical complement pathway. Immune complex formation and the binding of haptens, natural antigens or bivalent affinity labels were unaltered by complete removal of oligosaccharides from IgG. Antigen binding sites were similarly unaltered after acidic or basic buffer treatments followed by reneutralization. Removal of oligosaccharides with .**beta**.-**aspartyl** N-acetylglucosamido **hydrolase** or incubation at pH < 3.5 or > 10.5 (followed by neutralization to pH 7.0) irreversibly altered the abilities of rabbit IgG antibodies to interact with C1q. This lack of interaction with C1q was due to a lack of C1q binding and not due to an anticomplementary action of deglycosylated or pH-shifted IgG. Inhibition of C1q binding also correlated with an inability of several antibody populations to facilitate complement-mediated cytolysis. IgG structural alterations induced by charge perturbation and, to a minor extent, by deglycosylation could be detected by UV difference spectroscopy. Results were discussed in terms of the possible roles that Ig-linked oligosaccharides might play in facilitating IgG binding by C1q.

L8 ANSWER 39 OF 56 MEDLINE

ACCESSION NUMBER: 81086988 MEDLINE
DOCUMENT NUMBER: 81086988
TITLE: Purification by affinity chromatography using amastatin and properties of aminopeptidase A from pig kidney.
AUTHOR: Tobe H; Kojima F; Aoyagi T; Umezawa H
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1980 Jun 13) 613 (2) 459-68.
Journal code: A0W. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198105
AB 1. Amastatin, a specific inhibitor of aminopeptidase A (L-alpha-**aspartyl**(L-alpha-glutamyl)-peptide **hydrolase**, EC 3.4.11.7), was linked to an agarose matrix and by this affinity chromatography aminopeptidase A of pig kidneys was purified as a single protein shown by acrylamide gel electrophoresis. 2. Aminopeptidase A which was purified 710-fold, hydrolyzed only acidic amino acid **beta**-naphthylamide. The optimum pH and the optimum temperature was 7.5 and 45-50 degrees C, respectively. 3. The molecular weight was approx. 300 000 as determined by Sephadex G-200 gel filtration. 4. The activity of aminopeptidase A was not affected by sulfhydryl agents, S-S dissociating agents and serine proteinase inhibitor, but was inhibited strongly by metal chelating agents, and enhanced by alkaline earth metals. 5. Amastatin inhibited aminopeptidase A in a competitive manner with L-glutamic acid **beta**-naphthylamide, and the Ki value was calculated to be 2.5 x 10(-7) M. The inhibitory effect of amastatin on aminopeptidase A was not reversed by addition of Ca2+.

L8 ANSWER 40 OF 56 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 79152696 MEDLINE
DOCUMENT NUMBER: 79152696
TITLE: Quantitative determination of 4-N-2-acetamido-2-deoxy-beta-D-glucopyranosyl-L-asparagine in the urine of patients with aspartylglycosaminuria by gas-liquid chromatography.
AUTHOR: Maury P
SOURCE: JOURNAL OF LABORATORY AND CLINICAL MEDICINE, (1979 May) 93 (5) 718-23.
Journal code: IVR. ISSN: 0022-2143.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 197908
AB A specific and sensitive method for the quantitative ;determination

of glcNAc-Asn in the urine of patients with inherited deficiency of the lysosomal **hydrolase** N-**aspartyl-beta** -glucosaminidase is reported. The method is based on GLC assay of GlcNAc-Asn as its methylated derivative and requires 100 microliter of urine. The mean urinary excretion of GlcNAc-Asn in 14 AGU patients was 0.99 mmol/24 hr (range 0.15 to 1.88). Young patients had similar urinary levels of GlcNAc-Asn to those of the older ones when the results were calculated on the basis of creatinine excretion. Mass fragmentographic analysis revealed the presence of minimal amounts of GlcNAc-Asn in normal urine also. In four of the eight normal subjects studied, a rough quantitative estimation was feasible; the urinary output of GlcNAc-Asn in these subjects ranged from approximately 0.001 to 0.01 mmol/24 hr.

L8 ANSWER 41 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1979:78804 BIOSIS
DOCUMENT NUMBER: BR17:18804
TITLE: GLYCO PROTEIN STORAGE DISEASE.
AUTHOR(S): ISENBERG J N
SOURCE: WALBORG, EARL F., JR., (ED.). ACS (AMERICAN CHEMICAL SOCIETY) SYMPOSIUM SERIES, NO. 80. GLYCOPROTEINS AND GLYCOLIPIDS IN DISEASE PROCESSES. 175TH MEETING. ANAHEIM, CALIF., USA, MAR. 14-15, 1978. XV+480P. ILLUS. AMERICAN CHEMICAL SOCIETY: WASHINGTON, D.C., USA, (1978) 122-134.
ISBN: 0-8412-0452-7.
FILE SEGMENT: BR; OLD
LANGUAGE: Unavailable

L8 ANSWER 42 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1979:134998 BIOSIS
DOCUMENT NUMBER: BA67:14998
TITLE: 1 ASPARTAMIDO-**BETA**-N-ACETYL GLUCOSAMINE AND AMIDO **HYDROLASE** PURIFICATION KINETICS AND ITS ROLE IN **ASPARTYL** GLUCOSAMINURIA.
AUTHOR(S): DUGAL B; DUGAL R
CORPORATE SOURCE: INST. BIOL. GEOL., UNIV. TROMSO, P.O. BOX 790, 9001 TROMSO, NORW.
SOURCE: J MOL MED, (1978) 3 (1), 7-28.
CODEN: JMMEDM. ISSN: 0377-046X.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The distribution, intracellular localization, purification and kinetics of 1-aspartamido-.beta.-N-acetylglucosamine amidohydrolase (aspartyl-glucosylaninase, EC 3.5.1.26) are described. Its role in aspartylglucosaminuria is discussed.

L8 ANSWER 43 OF 56 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 76120459 MEDLINE

DOCUMENT NUMBER: 76120459
TITLE: Characterization of two glycoasparagines isolated from the urine of patients with aspartylglycosylaminuria (AGU).
AUTHOR: Sugahara K; Funakoshi S; Funakoshi I; Aula P; Yamashina I
SOURCE: JOURNAL OF BIOCHEMISTRY, (1975 Oct) 78 (4) 673-8. Journal code: HIF. ISSN: 0021-924X.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197606
AB Two major glycoasparagines (2-acetamido-N-(4'-L-**aspartyl**)-2-deoxy-**beta**-D-glycosylamines) were isolated from the urine of patients with aspartylglycosylaminuria (AGU). They were composed of equimolar amounts of sialic acid, galactose, glucosamine, and aspartic acid. They were isomeric with respect to the position of sialic acid attachment, since they produced the same glycoasparagine on incubation with the neuraminidase from Clostridium perfringens. The structure of the resulting sialic acid-free glycoasparagine was determined as **beta**-Gal-(1 leads to 4)-**beta**-GlcNAc-Asn based on the following findings. It produced galactose on incubation with **beta**-galactosidase, and N-acetyllactosamine and aspartic acid on incubation with 4-L-aspartylglycosylamine amindo **hydrolase**.

L8 ANSWER 44 OF 56 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 76131728 MEDLINE
DOCUMENT NUMBER: 76131728
TITLE: [Determination of the transferase activity of L-asparaginase].
Opredelenie transferaznoi aktivnosti L-asparaginazy.
AUTHOR: Buka M R; Zhagat R A; Kirstukas I P; Chepuka L Y
SOURCE: VOPROSY MEDITSINSKOI KHIMII, (1975 Sep-Oct) 21 (5) 548-52. Journal code: XIQ. ISSN: 0042-8809.
PUB. COUNTRY: USSR
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197606
AB A method for determination of the transferase activity of 1-asparaginase in presence of hydroxylamine is developed. The optimally determined quantity of the enzyme was from 0.7 to 20 i. u. The conditions optimal for the enzymatic reaction and for quantitative estimation of 1-**aspartyl-beta**-hydroxamic acid were studied. The transferase and **hydrolase**

activities of 1-asparaginase from E. coli were compared. The enzyme catalyzed at equal rates hydrolysis and hydroxylaminolysis of 1-asparagine.

L8 ANSWER 45 OF 56 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 76024818 MEDLINE
DOCUMENT NUMBER: 76024818
TITLE: Aminopeptidases of Bacillus subtilis.
AUTHOR: Desmond E P; Starnes W L; Behal F J
SOURCE: JOURNAL OF BACTERIOLOGY, (1975 Oct) 124 (1) 353-63.
Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197602

AB Three enzymes with L- and one enzyme with D-aminopeptidase (EC 3.4.11; alpha-aminoacyl peptide **hydrolase**) activity have been separated from each other and partially purified from Bacillus subtilis 168 W.T., distinguished with respect to their molecular weights and catalytic properties, and studied in relation to the physiology of this bacterium. One L-aminopeptidase, designated aminopeptidase I, has a molecular weight of 210,000 +/- 20,000, is produced early in growth, and hydrolyzes L-alanyl-**beta**-naphthylamide most rapidly. Another, designated aminopeptidase II, molecular weight 67,000 +/- 10,000, is also produced early in growth and hydrolyzes L-lysyl-**beta**-naphthylamide most rapidly. A third, aminopeptidase III, molecular weight 228,000 +/- 20,000, is produced predominantly in early stationary phase and most efficiently utilizes L-alpha-**aspartyl-beta**-naphthylamide as substrate. The synthesis of aminopeptidase III in early stationary phase suggests that selective catabolism of peptides occurs at this time, perhaps related to the cessation of growth or the onset of early sporulation-associated events. A D-aminopeptidase which hydrolyzes the carboxyl-blocked dipeptide D-alanyl-D-alanyl-**beta**-naphthylamide (as well as D-alanyl-**beta**-naphthylamide and D-alanyl-D-alanyl-D-alanine) has also been identified, separated from aminopeptidase II, and purified 170-fold. D-Aminopeptidase, molecular weight 220,000 +/- 20,000, is localized predominantly in the cell wall and periplasm of the organism. This evidence and the variation of the activity during the growth cycle suggest an important function in cell wall or peptide antibiotic metabolism.

L8 ANSWER 46 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1975:216279 BIOSIS
DOCUMENT NUMBER: BA60:46275
TITLE: THE LINKAGE OF CORNEAL KERATAN SULFATE TO PROTEIN.
AUTHOR(S): BAKER J R; CIFONELLI J A; RODEN L
Searcher : Shears 308-4994

SOURCE: CONNECT TISSUE RES, (1975) 3 (2-3), 149-156.
CODEN: CVTRBC. ISSN: 0300-8207.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable

L8 ANSWER 47 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1975:133907 BIOSIS
DOCUMENT NUMBER: BA59:33907
TITLE: THE AMINO-ACID SEQUENCE AND THE PEPTIDE CARBOHYDRATE LINKAGE OF GP-I-A AND GP-I-B GLYCO PEPTIDES FROM RHIZOPUS-JAVANICUS SACCHAROGENIC AMYLASE.
AUTHOR(S): WATANABE K; FUKIMBARA T
SOURCE: AGRIC BIOL CHEM, (1974) 38 (9), 1643-1647.
CODEN: ABCHA6. ISSN: 0002-1369.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable

L8 ANSWER 48 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1975:174867 BIOSIS
DOCUMENT NUMBER: BA60:4863
TITLE: CARBOHYDRATE COMPONENTS OF GLUCOSE OXIDASE FROM PENICILLIUM-VITALE.
AUTHOR(S): BOGDANOV V P; ABALIKHINA T A; CHUKHROVA A I; MOROZKIN A D; DEGTYAR R G; KAVERZNEVA E D
SOURCE: BIOKHIMIYA, (1974) 39 (4), 771-777.
CODEN: BIOHAO. ISSN: 0006-307X.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable

L8 ANSWER 49 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1975:13328 BIOSIS
DOCUMENT NUMBER: BR11:13328
TITLE: CULTURED SKIN FIBROBLASTS IN DISORDERS OF GLYCO PROTEIN CATABOLISM AND I CELL DISEASE.
AUTHOR(S): AUTIO S; AULA P; NAENTOE V
SOURCE: Dev. Med. Child Neurol., (1974) 16 (3), 376-378.
CODEN: DMCNAW. ISSN: 0012-1622.
FILE SEGMENT: BR; OLD
LANGUAGE: Unavailable

L8 ANSWER 50 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1974:171525 BIOSIS
DOCUMENT NUMBER: BA58:1219
TITLE: ENZYMES HYDROLYZING L LEUCYL-BETA NAPHTHYLAMIDE AND ALPHA-L ASPARTYL-P-NITRO ANILIDE IN HUMAN PLACENTA DURING DEVELOPMENT.
AUTHOR(S): WARWAS M; DOBRYSZYCKA W; SWARD J
SOURCE: ENZYME (BASEL), (1972-1973) 14 (6), 340-347.
CODEN: ENZYBT. ISSN: 0013-9432.

FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable

L8 ANSWER 51 OF 56 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 74032080 EMBASE
DOCUMENT NUMBER: 1974032080
TITLE: Aspartylglucosaminuria: deficiency of aspartylglucosaminidase in cultured fibroblasts of patients and their heterozygous patients.
AUTHOR: Aula P.; Nanto V.; Laipio M.L.; Autio S.
CORPORATE SOURCE: Child. Hosp., Univ. Helsinki, Finland
SOURCE: Clinical Genetics, (1973) 4/3 (297-300).
CODEN: CLGNAY
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
007 Pediatrics and Pediatric Surgery
029 Clinical Biochemistry
008 Neurology and Neurosurgery
LANGUAGE: English
AB Aspartylglucosaminuria (AGU) is a genetic lysosomal storage disorder which probably affects the metabolism of glycoproteins. Earlier studies have shown a deficiency of a lysosomal **hydrolase**, N **aspartyl** .**beta**. glucosaminidase in the serum and seminal fluid, as well as in the brain and liver tissues of the patients. The present studies demonstrated a very low activity of N **aspartyl** .**beta**. glucosaminidase in cultured skin fibroblasts from AGU patients. The fibroblasts of the parents of the patients had a moderately low enzyme activity when compared with control cultures. Thus, demonstration of the enzyme defect in fibroblasts offers possibilities both for detection of heterozygotes and for prenatal diagnosis of AGU.

L8 ANSWER 52 OF 56 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 73222498 MEDLINE
DOCUMENT NUMBER: 73222498
TITLE: Presence of **beta-aspartyl** N-acetyl glucosamine amido **hydrolase** in mammalian spermatozoa.
AUTHOR: Bhalla V K; Tillman W L; Williams W L
SOURCE: JOURNAL OF REPRODUCTION AND FERTILITY, (1973 Jul) 34 (1) 137-9.
Journal code: JWN. ISSN: 0022-4251.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197311

L8 ANSWER 53 OF 56 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 74130963 EMBASE
DOCUMENT NUMBER: 1974130963
TITLE: Enzymes hydrolysing L leucyl .beta. naphthylamide and .alpha. L aspartyl p nitroanilide in human placenta during development.
AUTHOR: Warwas M.; Dobryszycka W.; Sward J.
CORPORATE SOURCE: Dept. Clin. Investigat., Biol. Pharmaceut. Inst., Med. Acad., Wroclaw, Poland
SOURCE: Enzyme, (1972) 14/6 (340-347).
CODEN: ENZYBT
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
021 Developmental Biology and Teratology
016 Cancer
LANGUAGE: English
AB Activities of L leucyl .**beta**. naphthylamidase (aminoacyl naphthylamide amidohydrolase EC 3.4.1.1), and .alpha. L **aspartyl** p nitroanilide **hydrolase** of placenta and related tissues were determined in 4 periods of the development of human placenta. It is shown that both enzymes were the most active in placentas originating from the period of growth and differentiation, i.e., between the 14th and 28th wk of gestation. Three zones of L leucyl .**beta**. naphthylamidase were demonstrated by polyacrylamide gel electrophoresis in extracts of placenta, and four zones in membranes. Placenta was found to contain one zone of .alpha. L **aspartyl** naphthylamidase, membranes were devoid of this activity.

L8 ANSWER 54 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1973:14936 BIOSIS
DOCUMENT NUMBER: BR09:14936
TITLE: DEMONSTRATION OF **BETA ASPARTYL** N ACETYL GLUCOSAMINE **HYDROLASE** IN BOAR SPERM ACROSOMAL EXTRACT.
AUTHOR(S): BHALLA V K
SOURCE: Biol. Reprod., (1972) 7 (1), 101.
CODEN: BIREBV. ISSN: 0006-3363.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: Unavailable

L8 ANSWER 55 OF 56 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1971:109448 BIOSIS
DOCUMENT NUMBER: BA52:19448
TITLE: THE PURIFICATION AND PROPERTIES OF A **BETA ASPARTYL**-N-ACETYL GLUCOSYLAMINE AMIDO **HYDROLASE** FROM HEN OVIDUCT.
AUTHOR(S): TARENTINO A L; MALEY F
SOURCE: ARCH BIOCHEM BIOPHYS, (1969) 130 (1-2), 295-303.

CODEN: ABBIA4. ISSN: 0003-9861.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable

L8 ANSWER 56 OF 56 CONFSCI COPYRIGHT 1999 CSA
ACCESSION NUMBER: 91:52084 CONFSCI
DOCUMENT NUMBER: 92020526
TITLE: Purification, characterization, cloning and expression of bovine liver **aspartyl-beta -hydroxylase**
AUTHOR: Friedman, P.A.
CORPORATE SOURCE: Merck Sharp & Dohme Res. Lab.
SOURCE: American Society of Hematology, 1101 Connecticut Avenue NW, Suite 700, Washington, DC 20036, USA. Telephone: (202) 857-1118. Fax: (202) 223-4579.. Meeting Info.: 914 5026: 33rd Annual Meeting and Exposition of the American Society of Hematology (9145026). Denver, CO (USA). 6-10 Dec 1991.
DOCUMENT TYPE: Conference
FILE SEGMENT: DCCP
LANGUAGE: UNAVAILABLE

FILE 'CAPLUS' ENTERED AT 15:22:24 ON 22 OCT 1999
L9 7 S ASPARAGINYL(S)(HYDROXYLASE OR HYDROLASE)
L10 0 S L9 NOT L6

FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS' ENTERED AT 15:22:49 ON 22 OCT 1999
L11 39 S L9
L12 24 S L11 NOT L8
L13 22 S L12 AND BETA
L14 9 DUP REM L13 (13 DUPLICATES REMOVED)

=> d 1-9 ibib abs

L14 ANSWER 1 OF 9 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:491983 SCISEARCH
THE GENUINE ARTICLE: ZV209
TITLE: Post-translational modifications required for coagulation factor secretion and function
AUTHOR: Kaufman R J (Reprint)
CORPORATE SOURCE: UNIV MICHIGAN, SCH MED, DEPT BIOL CHEM, HOWARD HUGHES MED INST, ANN ARBOR, MI 48109 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: THROMBOSIS AND HAEMOSTASIS, (JUN 1998) Vol. 79, No. 6, pp. 1068-1079.
Publisher: F K SCHATTAUER VERLAG GMBH, P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY.
ISSN: 0340-6245.

DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 139

L14 ANSWER 2 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 1
ACCESSION NUMBER: 1998:477969 BIOSIS
DOCUMENT NUMBER: PREV199800477969
TITLE: The five cysteine residues located in the active site region of bovine aspartyl (**asparaginyl**) **beta-hydroxylase** are not essential for catalysis.
AUTHOR(S): McGinnis, Kathleen (1); Ku, Gregory M.; Fu, Jeffrey; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: (1) c/o Jeffrey Fu, Merck and Co. Inc., WP37T2-3, West Point, PA 19486 USA
SOURCE: Biochimica et Biophysica Acta, (Sept. 8, 1998) Vol. 1387, No. 1-2, pp. 454-456.
ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
AB In previous chemical modification studies on bovine aspartyl (**asparaginyl**) **beta-hydroxylase**, cysteines were implicated as critical catalytic residues. Using site-directed mutagenesis, the five cysteine residues located in a highly conserved region of the enzyme identified as the active site were individually mutated to alanine. Substitutions at cysteine 637, 644, 656, 681, and 696 resulted in active mutant enzymes indicating that these residues are not required for catalysis.

L14 ANSWER 3 OF 9 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 97:360088 SCISEARCH
THE GENUINE ARTICLE: WV419
TITLE: Cellular overexpression of human **asparaginyl** /aspartyl **beta-hydroxylase** (HAAH) in gastric and colon cancers
AUTHOR: Nishimaki H (Reprint); Nishiyama M; Wands J R; Tanaka T
CORPORATE SOURCE: MGH CANC CTR, CHARLESTOWN, MA; JIKEI UNIV, SCH MED, DEPT INTERNAL MED, TOKYO, JAPAN; JIKEI UNIV, SCH MED, DEPT BIOCHEM, TOKYO, JAPAN
COUNTRY OF AUTHOR: USA; JAPAN
SOURCE: GASTROENTEROLOGY, (APR 1997) Vol. 112, No. 4, Supp. [S], pp. A628-A628.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.
ISSN: 0016-5085.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN

LANGUAGE: English
REFERENCE COUNT: 1

L14 ANSWER 4 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 2
ACCESSION NUMBER: 1996:237846 BIOSIS
DOCUMENT NUMBER: PREV199698785975
TITLE: Site-directed mutagenesis of residues in a conserved region of bovine aspartyl (**asparaginyl**) **beta-hydroxylase**: Evidence that histidine 675 has a role in binding Fe-2.
AUTHOR(S): McGinnis, Kathleen (1); Ku, Gregory M.; Vandusen, William J.; Fu, Jeffrey; Garsky, Victor; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: (1) c/o Jeffrey Fu at Merck Res. Lab., WP26B-1116, West Point, PA 19486 USA
SOURCE: Biochemistry, (1996) Vol. 35, No. 13, pp. 3957-3962. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The roles in catalysis of several residues in bovine aspartyl (**asparaginyl**) **beta-hydroxylase** that are located in a region of homology among alpha-ketoglutarate-dependent dioxygenases were investigated using site-directed mutagenesis. Previous studies have shown that when histidine 675, an invariant residue located in this highly conserved region, was mutated to an alanine residue, no enzymatic activity was detected. A more extensive site-directed mutagenesis study at position 675 has been undertaken to define the catalytic role of this essential residue. The partial **hydroxylase** activity observed with some amino acid replacements for histidine 675 correlates with the potential to coordinate metals and not with size, charge, or hydrophobic character. Furthermore, the increase in K-m for Fe-2+ observed with the H675D and H675E mutant enzymes can account for their partial activities relative to wild type. No significant changes in the K-m for alpha-ketoglutarate (at saturating Fe-2+) or V-max were observed for these mutants. These results support the conclusion that histidine 675 is specifically involved in Fe-2+ coordination. Further site-directed mutagenesis of other highly conserved residues in the vicinity of position 675 demonstrates the importance of this region of homology in catalysis for Asp (Asn) **beta-hydroxylase** and, by analogy, other alpha-ketoglutarate-dependent dioxygenases.

L14 ANSWER 5 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 3
ACCESSION NUMBER: 1996:511225 BIOSIS
DOCUMENT NUMBER: PREV199699233581
TITLE: Overexpression of human aspartyl(**asparaginyl**)**beta-hydroxylase** in hepatocellular carcinoma and cholangiocarcinoma.

AUTHOR(S): Lavaissiere, Laurent; Jia, Steve; Nishiyama, Masaki; De La Monte, Suzanne; Stern, Andrew M.; Wands, Jack R.; Friedman, Paul A. (1)
CORPORATE SOURCE: (1) DuPont Merck Res. Lab., Experimental Station E4002426, Wilmington, DE 19880-0400 USA
SOURCE: Journal of Clinical Investigation, (1996) Vol. 98, No. 6, pp. 1313-1323.
ISSN: 0021-9738.
DOCUMENT TYPE: Article
LANGUAGE: English
AB To characterize genes that become upregulated with malignant transformation of human hepatocytes, a library of monoclonal antibodies was produced against the FOCUS hepatocellular carcinoma cell line. Antibody FB-50 reacted with an antigen that was highly expressed in 4 of 10 primary hepatocellular carcinomas, in all 20 cholangiocarcinomas we studied, and in a variety of transformed cell lines. This antigen was also highly expressed in neoplastic epithelial cells of breast and colon carcinomas in contrast to its low level of expression in normal hepatocytes and in nonneoplastic epithelial cells. Among the normal adult tissues studied, high levels were observed only in proliferating trophoblastic cells of the placenta and in adrenal glands. A 636-bp partial cDNA, isolated from a gamma-GT11 expression library generated with HepG2 human hepatoblastoma cells, and a complete cDNA, generated by reverse transcriptase-PCR, identified the antigen as the human form of aspartyl(**asparaginyl**)**beta-hydroxylase**.
This enzyme catalyzes posttranslational hydroxylation of **beta** carbons of specific aspartyl and **asparaginyl** residues in EGF-like domains of certain proteins. Analyses of extracts prepared from several human tumor cell lines compared to their normal tissue counterparts indicate that the increase in **hydroxylase**, apprx 10-fold, is controlled at the level of transcription and the protein is expressed in an enzymatically active form. In similar analyses, comparing hepatocellular carcinomas to adjacent uninvolved liver from five patients, enzymatic activity was much higher in the tumor tissue from the four patients whose immunoblots revealed increased **hydroxylase** protein in the malignant tissue. EGF repeats in the extracellular domain of Notch or its homologs contain the consensus sequence for hydroxylation. Deletion mutants lacking this domain are gain-of-function mutants, suggesting that the domain modulates signal transduction by the cytoplasmic domain. While the function imparted by **beta** hydroxylation is unknown, our studies raise the possibility that **beta** hydroxylation is regulated in proteins like the mammalian Notch homologs, whose cytoplasmic domains have been shown to be oncogenic.

L14 ANSWER 6 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1994:404846 BIOSIS

DOCUMENT NUMBER: PREV199497417846
TITLE: A fully active catalytic domain of bovine aspartyl (**asparaginyl**) **beta-hydroxylase** expressed in Escherichia coli: Characterization and evidence for the identification of an active-site region in vertebrate alpha-ketoglutarate-dependent dioxygenases.
AUTHOR(S): Jia, Steve; McGinnis, Kathleen (1); Vandusen, William J.; Burke, Carl J.; Kuo, Amy; Griffin, Patrick R.; Sardana, Mohinder K.; Elliston, Keith O.; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: (1) Merck Res. Laboratories, WP26-207, West Point, PA 19486 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994) Vol. 91, No. 15, pp. 7227-7231.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The alpha-ketoglutarate-dependent dioxygenase aspartyl (**asparaginyl**) **beta-hydroxylase** (EC 1.14.11.16) specifically hydroxylates one aspartic or asparagine residue in certain epidermal growth factor-like domains of a number of proteins. The expression in Escherichia coli, purification, characterization of a fully active catalytic domain, and evidence for the identification of an active-site region of this enzyme are described. Sequence alignment analyses among the vertebrate alpha-ketoglutarate-dependent dioxygenases and chemical modification studies were undertaken aimed at locating specific regions of 52-kDa recombinant aspartyl (**asparaginyl**) **beta-hydroxylase** involved in substrate binding and/or catalysis. Based upon these studies, an alignment of the C-terminal regions of prolyl and lysyl **hydroxylase** and of aspartyl (**asparaginyl**) **beta-hydroxylase** is proposed. When histidine-675, an invariant residue located in a region of homology within this alignment, was mutated to an alanine residue in aspartyl (**asparaginyl**) **beta-hydroxylase** (H675A), no enzymatic activity was detected. Chemical modification studies show that the wild-type protein is protected from iodo(14C)acetamide labeling by Fe-2+/alpha-ketoglutarate whereas the H675A mutant protein is not, suggesting that this mutant does not bind Fe-2+/alpha-ketoglutarate.

L14 ANSWER 7 OF 9 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 94:265839 SCISEARCH
THE GENUINE ARTICLE: NH516
TITLE: IDENTIFICATION OF ACTIVE-SITE RESIDUES IN BOVINE ASPARTYL (**ASPARAGINYL**) **BETA-HYDROXYLASE** BY SITE-DIRECTED MUTAGENESIS

AUTHOR: MCGINNIS K (Reprint); VANDUSEN W J; KU G; STERN A M; FRIEDMAN P A
CORPORATE SOURCE: MERCK SHARP & DOHME LTD, W POINT, PA, 19486
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (19 APR 1994) Vol. 8, No. 7, pp. A1369.
ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: No References

L14 ANSWER 8 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 5
ACCESSION NUMBER: 1992:409920 BIOSIS
DOCUMENT NUMBER: BA94:73120
TITLE: CDNA CLONING AND EXPRESSION OF BOVINE ASPARTYL **ASPARAGINYL BETA HYDROXYLASE**.
AUTHOR(S): JIA S; VANDUSEN W J; DIEHL R E; KOHL N E; DIXON R A F; ELLISTON K O; STERN A M; FRIEDMAN P A
CORPORATE SOURCE: MERCK SHARP DOHME RESEARCH LABORATORIES, WP44-204, WEST POINT, PA. 19486.
SOURCE: J BIOL CHEM, (1992) 267 (20), 14322-14327.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Aspartyl (**asparaginyl**) .**beta**.-**hydroxylase** which specifically hydroxylates 1 Asp or Asn residue in certain epidermal growth factor-like domains of a number of proteins, has been previously purified to apparent homogeneity from detergent-solubilized bovine liver microsomes (Wang, Q., VanDusen, W. J., Petroski, C. J., Garsky, V. M., Stern, A. M. and Friedman, P. A. (1991) J. Biol. Chem. 266, 14004-14010). Three oligonucleotides, corresponding to three amino acid sequences of the purified **hydroxylase**, were used to screen bovine cDNA libraries. Several overlapping positive cDNA clones containing a full length open reading frame of 754 amino acids encoding a 85-kDa protein were isolated, and a cDNA, containing the full length open reading frame, was constructed from two of these clones. The resulting clone was then transcribed and translated in vitro to produce recombinant protein which possessed Asp .**beta**.-**hydroxylase** activity. These results constitute proof that the protein purified from bovine liver is an Asp .**beta**.-**hydroxylase**. Comparisons of deduced amino acid sequences of two other .alpha.-ketoglutarate-dependent dioxygenases, prolyl-4-**hydroxylase** and lysyl **hydroxylase**, with that of Asp .**beta**.-**hydroxylase** showed no significant homologies. Indeed, Asp .**beta**.-**hydroxylase** appears to be unique as no striking homology was found with known

protein sequences. Furthermore, structural predictions derived from the deduced amino acid sequence are in accord with earlier Stokes' radius and sedimentation coefficient determinations of the enzyme, suggesting that the enzyme contains a relatively compact carboxyl-terminal catalytic domain and an extended amino terminus. This amino-terminal region has a potential transmembrane type II signal-anchor domain that could direct the catalytic domain into the lumen of the endoplasmic reticulum.

L14 ANSWER 9 OF 9 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1993:118728 BIOSIS
DOCUMENT NUMBER: PREV199395062828
TITLE: Invertebrate aspartyl/**asparaginyl beta-hydroxylase**: Potential modification of endogenous epidermal growth factor-like modules.
AUTHOR(S): Monkovic, Don D.; Vandusen, William J.; Petroski, Christopher J.; Garsky, Victor M.; Sardana, Mohinder K.; Zavodszky, Peter; Stern, Andrew M.; Friedman, Paul A.
CORPORATE SOURCE: Merck Res. Lab., West Point, Pa. 19486
SOURCE: Biochemical and Biophysical Research Communications, (1992) Vol. 189, No. 1, pp. 233-241.
ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB An invertebrate alpha-ketoglutarate-dependent aspartyl/**asparaginyl beta-hydroxylase**, which posttranslationally hydroxylates specific aspartyl or **asparaginyl** residues within epidermal growth factor-like modules, was identified, partially purified and characterized. Preparations derived from two insect cell lines catalyzed the hydroxylation of the expected **asparaginyl** residue within a synthetic epidermal growth factor-like module. This activity was found to be similar to that of the purified mammalian aspartyl/**asparaginyl beta-hydroxylase** with respect to cofactor requirements, stereochemistry and substrate sequence specificity. Furthermore, recombinant human C1 hivin r, expressed in an insect cell-derived baculovirus expression system, was also found to be hydroxylated at the expected **asparaginyl** residue. Thus, these results establish the potential for invertebrate aspartyl/**asparaginyl** hydroxylation. Since several invertebrate proteins known to be required for proper embryonic development contain a putative consensus sequence that may be required for hydroxylation, the studies presented here provide the basis for further investigations concerned with identifying hydroxylated invertebrate proteins and determining their physiologic function.

FILE 'USPATFULL' ENTERED AT 15:24:05 ON 22 OCT 1999
L15 4 S L6
L16 7 S L9
L17 8 S L15 OR L16

L17 ANSWER 1 OF 8 USPATFULL

ACCESSION NUMBER:	1999:124922 USPATFULL
TITLE:	Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation
INVENTOR(S):	Hanauske-Abel, Hartmut M., Edgewater, NJ, United States McCaffrey, Timothy A., New York, NY, United States Grady, Robert Walter, Kinnelon, NJ, United States
PATENT ASSIGNEE(S):	Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5965586	19991012
APPLICATION INFO.:	US 1997-991758	19971216 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1995-377137, filed on 20 Jan 1995, now patented, Pat. No. US 5789426	
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Krass, Frederick	
LEGAL REPRESENTATIVE:	Nixon Peabody LLP	
NUMBER OF CLAIMS:	27	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	8 Drawing Figure(s); 11 Drawing Page(s)	
LINE COUNT:	1302	

AB The present invention relates to a method of treating a patient with a fibrotic or fibroproliferative disorder and a method of suppressing formation of collagen and collagen-like substances or biosynthesis of procollagen in living systems by administering to a patient or living system, respectively, an effective amount of a compound of Formulae (I) or (II) and derivatives thereof: ##STR1## R.sub.1, R.sub.2, R.sub.3, and R.sub.4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms, an aryl, aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

INCL INCLM: 514/348.000
INCLS: 514/002.000
NCL NCLM: 514/348.000
NCLS: 514/002.000

Searcher : Shears 308-4994

L17 ANSWER 2 OF 8 USPATFULL
ACCESSION NUMBER: 1999:124921 USPATFULL
TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation
INVENTOR(S): Hanauske-Abel, Hartmut M., Edgewater, NJ, United States
McCaffrey, Timothy A., New York, NY, United States
Grady, Robert Walter, Kinnelon, NJ, United States
PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5965585	19991012
APPLICATION INFO.:	US 1997-866998	19970530 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1995-377137, filed on 20 Jan 1995, now patented, Pat. No. US 5789426
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Jones, Dwayne C.
LEGAL REPRESENTATIVE: Nixon, Hargrave, Devans & Doyle LLP
NUMBER OF CLAIMS: 18
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 1260
AB The present invention relates to a method of treating a patient with a fibrotic or fibroproliferative disorder and a method of suppressing formation of collagen and collagen-like substances or biosynthesis of procollagen in living systems by administering to a patient or living system, respectively, an effective amount of a compound of Formulae (I) or (II) and derivatives thereof: ##STR1## R.sub.1, R.sub.2, R.sub.3, and R.sub.4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms, an aryl, aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

INCL INCLM: 514/348.000
NCL NCLM: 514/348.000

L17 ANSWER 3 OF 8 USPATFULL
ACCESSION NUMBER: 1998:157194 USPATFULL
TITLE: Method of inhibiting viral replication in eukaryotic cells and of inducing apoptosis of virally-infected cells
INVENTOR(S): Hanauske-Abel, Hartmut M., Edgewater, NJ, United States

Grady, Robert Walter, Kinnelon, NJ, United States
Hanauske, Axel, Wolratshausen, Germany, Federal Republic of
Andrus, Linda, New York, NY, United States
Szabo, Paul, Linden, NJ, United States
PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5849587	19981215
APPLICATION INFO.:	US 1995-488811	19950609 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Degen, Nancy
ASSISTANT EXAMINER: Yucel, Irem
LEGAL REPRESENTATIVE: Nixon, Hargrave, Devans & Doyle LLP
NUMBER OF CLAIMS: 61
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 31 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 1589
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention is directed to methods which employ inhibition of the post-translational hypusine formation in the intracelluar protein eIF-5A, for the purpose of suppressing infections by viruses that parasitize eIF-5A so as to promote their own replication. Intentional inhibition of the post-translational formation of hypusine in infected host cells with compounds generically termed `hypusine inhibitors` not only selectively suppresses the production of viral proteins and of infectious viral particles, but also causes, particularly after hypusine inhibitor withdrawal, apoptosis in such virally-infected cells. Each of these methods, respectively, involves administering, to eukaryotic cells, tissues, or individuals, an agent which blocks the post-translational intracellular formation of hypusine, in an amount sufficient to: suppress biosynthesis of bioactive eIF-5A, suppress translational interaction of eIF-5A with viral elements of nucleic acid and/or protein structure, inhibit biosynthesis of viral proteins of Rev-dependent lentiviruses or of viruses dependent on interaction of eIF-5A with viral elements of nucleic acid and/or protein structure, inhibit replication of Rev-dependent lentiviruses or of viruses dependent on interaction of eIF-5A with viral elements of nucleic acid and/or protein structure, and induce apoptosis of virally-infected cells. This agent can be a compound of Formulae I or II and derivatives thereof as follows: ##STR1## R.sub.1, R.sub.2, R.sub.3, and R.sub.4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms, an aryl, aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up

to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/372.300
INCLS: 435/375.000; 514/185.000
NCL NCLM: 435/372.300
NCLS: 435/375.000; 514/185.000

L17 ANSWER 4 OF 8 USPATFULL
ACCESSION NUMBER: 1998:92047 USPATFULL
TITLE: Method for the treatment of fibroproliferative disorders by application of inhibitors of protein hydroxylation
INVENTOR(S): Hanauske-Abel, Hartmut M., Edgewater, NJ, United States
McCaffrey, Timothy A., New York, NY, United States
Grady, Robert Walter, Kinnelon, NJ, United States
PATENT ASSIGNEE(S): Cornell Research Foundation, Inc., Ithaca, NY, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5789426	19980804
APPLICATION INFO.:	US 1995-377137	19950120 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Mullis, Jeffrey
LEGAL REPRESENTATIVE: Nixon, Hargrave, Devans & Doyle
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Figure(s); 11 Drawing Page(s)
LINE COUNT: 1309
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method of treating a patient with a fibrotic or fibroproliferative disorder and a method of suppressing formation of collagen and collagen-like substances or biosynthesis of procollagen in living systems by administering to a patient or living system, respectively, an effective amount of a compound of Formulae (I) or (II) and derivatives thereof: ##STR1## R.sub.1, R.sub.2, R.sub.3, and R.sub.4 each individually represent a hydrogen, an alkyl, alkenyl, or alkoxy group containing 1 to about 8 carbon atoms, an aryl, aralkyl, or cycloalkyl group containing about 5 to 12 carbon atoms, or a carboalkoxy or carbamyl group containing up to 8 carbon atoms, or a peptide or peptidomimetic moiety containing 10 to about 30 carbon atoms.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 514/348.000

INCLS: 546/296.000
NCL NCLM: 514/348.000
NCLS: 546/296.000

L17 ANSWER 5 OF 8 USPATFULL
ACCESSION NUMBER: 1998:57716 USPATFULL
TITLE: Aptamers specific for biomolecules and methods of making
INVENTOR(S): Griffin, Linda, Atherton, CA, United States
Albrecht, Glenn, Redwood City, CA, United States
Latham, John, Palo Alto, CA, United States
Leung, Lawrence, Hillsborough, CA, United States
Vermaas, Eric, Oakland, CA, United States
Toole, John J., Burlingame, CA, United States
PATENT ASSIGNEE(S): Gilead Sciences, Inc., Foster City, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5756291	19980526
APPLICATION INFO.:	US 1995-484192	19950607 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1992-934387, filed on 21 Aug 1992, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Bosse, Mark L.
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 8242
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for identifying oligomer sequences, optionally comprising modified base, which specifically bind target molecules such as serum proteins, kinins, eicosanoids and extracellular proteins is described. The method is used to generate aptamers that bind to serum Factor X, PDGF, FGF, ICAM, VCAM, E-selectin, thrombin, bradykinin, PGF2 and cell surface molecules. The technique involves complexation of the target molecule with a mixture of oligonucleotides containing random sequences and sequences which serve as primer for PCR under conditions wherein a complex is formed with the specifically binding sequences, but not with the other members of the oligonucleotide mixture. The complex is then separated from uncomplexed oligonucleotides and the complexed members of the oligonucleotide mixture are recovered from the separated complex using the polymerase chain reaction. The recovered oligonucleotides may be sequenced, and successive rounds of selection using complexation, separation, amplification and recovery can be employed. The oligonucleotides can be used for therapeutic and diagnostic purposes and for generating secondary

aptamers.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/006.000
INCLS: 536/023.100; 530/413.000; 935/077.000; 935/078.000
NCL NCLM: 435/006.000
NCLS: 530/413.000; 536/023.100

L17 ANSWER 6 OF 8 USPATFULL
ACCESSION NUMBER: 96:36467 USPATFULL
TITLE: Acyl-peptide hydrolase and methods of production and use
INVENTOR(S): Smith, John A., Brookline, MA, United States
PATENT ASSIGNEE(S): The General Hospital Corporation, Boston, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5512467	19960430
APPLICATION INFO.:	US 1993-91445	19930715 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1989-429935, filed on 1 Nov 1989, now patented, Pat. No. US 5268267 which is a continuation-in-part of Ser. No. US 1989-296996, filed on 13 Jan 1989, now abandoned which is a continuation-in-part of Ser. No. US 1987-87936, filed on 21 Aug 1987, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Wax, Robert A.
ASSISTANT EXAMINER: Prouty, Rebecca
LEGAL REPRESENTATIVE: Sterne, Kessler, Goldstein & Fox
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 11
NUMBER OF DRAWINGS: 26 Drawing Figure(s); 23 Drawing Page(s)
LINE COUNT: 1615
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention discloses the amino acid sequence of Acyl-Peptide Hydrolase. The invention relates to a DNA molecule encoded for Acyl-Peptide Hydrolase, a vector incorporating the molecule, and the use of such vector to transform a host. The invention further relates to the expression by the host to produce Acyl-Peptide Hydrolase, and the use of APH to catalyze the hydrolysis of an N.sup..alpha. -acetylated peptide or protein or the reaction between an N.sup..alpha. -acetyl amino acid donor and an acceptor protein with a free .alpha.-NH.sub.2 group. The invention further relates to the use of the Acyl-Peptide Hydrolase-encoding sequences in the detection of cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/172.300

INCLS: 435/240.200; 435/252.300; 435/212.000; 435/320.100; 435/254.200; 536/023.200
NCL NCLM: 435/488.000
NCLS: 435/212.000; 435/252.300; 435/254.200; 435/320.100; 435/358.000; 435/364.000; 536/023.100

L17 ANSWER 7 OF 8 USPATFULL
ACCESSION NUMBER: 93:102680 USPATFULL
TITLE: Method for diagnosing small cell carcinoma
INVENTOR(S): Smith, John A., Brookline, MA, United States
PATENT ASSIGNEE(S): The General Hospital Corporation, Boston, MA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5268267	19931207
APPLICATION INFO.:	US 1989-429935	19891101 (7)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1987-87936, filed on 21 Aug 1987, now abandoned And a continuation-in-part of Ser. No. US 1989-296996, filed on 13 Jan 1989, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Yarbrough, Amelia Burgess
LEGAL REPRESENTATIVE: Sterne, Kessler, Goldstein & Fox
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 23 Drawing Page(s)
LINE COUNT: 1640
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the use of the Acyl-Peptide Hydrolase-encoding sequences in the detection of cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/006.000
INCLS: 435/018.000; 435/172.300; 436/064.000; 436/813.000; 536/023.200; 935/078.000
NCL NCLM: 435/006.000
NCLS: 435/018.000; 436/064.000; 436/813.000; 536/023.200

L17 ANSWER 8 OF 8 USPATFULL
ACCESSION NUMBER: 92:18897 USPATFULL
TITLE: Asparaginyl endopeptidase, composition and use thereof
INVENTOR(S): Matsushita, Hideyuki, Muko, Japan
Kato, Ikunoshin, Uji, Japan
Abe, Yukichi, Sapporo, Japan
Ishii, Shin-ichi, Ichikawa, Japan
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto, Japan (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5094952	19920310	
APPLICATION INFO.:	US 1990-527729	19900523	(7)

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1989-297450	19891117

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Robinson, Douglas W.
ASSISTANT EXAMINER: Meller, Michael V.
LEGAL REPRESENTATIVE: Wenderoth, Lind & Ponack
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 2
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 249
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An asparaginyl endopeptidase which is specific for only an amide bond on the C-terminal side of an L-asparagine. Also disclosed is a method for the hydrolysis of an amide bond on the C-terminal side of an L-asparagine characterized by the use of an asparaginyl endopeptidase as well as a composition for use in the hydrolysis of an amide bond on the C-terminal side of an L-asparagine.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
INCL INCLM: 435/212.000
INCLS: 435/227.000; 435/228.000
NCL NCLM: 435/212.000
NCLS: 435/227.000; 435/228.000

Seq. IDs

FILE 'REGISTRY' ENTERED AT 15:25:06 ON 22 OCT 1999
L18 7 S APPEDNPVED | EEQQEVPPDT | DGPTGEPQQE | QENPDSSEPV/SQSP

FILE 'CAPLUS' ENTERED AT 15:25:54 ON 22 OCT 1999
L19 4 S L18
L20 0 S L19 NOT L6

FILE 'CANCERLIT' ENTERED AT 15:27:07 ON 22 OCT 1999
L21 3 S L6
L22 2 S L9
L23 3 S L21 OR L22

L23 ANSWER 1 OF 3 CANCERLIT
ACCESSION NUMBER: 97619115 CANCERLIT
DOCUMENT NUMBER: 97619115
TITLE: Detection beta-aspartyl(Asparaginyl) hydroxylation in Notch (Meeting abstract).
AUTHOR: Jia S; Ma J; Stern A M; Corman J; Blom K; Weinmaster

Searcher : Shears 308-4994

G; Friedman P A
CORPORATE SOURCE: Dupont Merck Research Laboratories, Wilmington, DE 19880.
SOURCE: Proc Annu Meet Am Assoc Cancer Res, (1997). Vol. 38, pp. A431.
ISSN: 0197-016X.
DOCUMENT TYPE: (MEETING ABSTRACTS)
FILE SEGMENT: ICDB; L
LANGUAGE: English
ENTRY MONTH: 199709

AB We have demonstrated (J Clin Invest; 98:1313 1996) in carcinomas of human liver, colon and breast, human **beta-aspartyl (asparaginyl) hydroxylase(HAAH)** activity is elevated more than 10 fold relative to normal tissue counterparts. These result raise the possibility that **HAAH** has a role in tumorigenesis; one hypothesis involves mammalian Notch homologues as the target substrates of **HAAH**. The extracellular domains of these proteins contain 20 EGF-like repeats having consensus sequences for hydroxylation. The level of hydroxylation may regulate ligand binding of the extracellular domains which may in turn regulate the activity of the cytoplasmic domains which are known to be oncogenic. Rat full length Notch was expressed in Mouse L cells and purified by immuno-affinity chromatography followed by SDS PAGE. The Notch was then transferred to PVDF membrane, hydrolyzed and then subjected to **beta** -hydroxyl aspartic acid determination by two independent methods, ion exchange HPLC and LC/MS/MS. By both methods, 10-20% of potential sites were found to be hydroxylated. Notch is thus hydroxylated at a level that could potentially increase in the cells with elevated **hydroxylase** activity. Our finding may be one mechanism of regulation of the Notch signal transduction pathway and thus may provide a functional link between **HAAH** and tumorigenesis.

L23 ANSWER 2 OF 3 CANCERLIT
ACCESSION NUMBER: 96420598 CANCERLIT
DOCUMENT NUMBER: 96420598
TITLE: Overexpression of human **aspartyl(asparaginyl)beta-hydroxylase** in hepatocellular carcinoma and cholangiocarcinoma.
AUTHOR: Lavaissiere L; Jia S; Nishiyama M; de la Monte S; Stern A M; Wands J R; Friedman P A
CORPORATE SOURCE: Molecular Hepatology Laboratory, Massachusetts General Hospital Cancer Center, Charlestown 02129, USA.
CONTRACT NUMBER: CA-35711 (CA)
AA-08169 (AA)
AA-02666 (AA)
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1996). Vol. 98,

No. 6, pp. 1313-23.
Journal code: HS7. ISSN: 0021-9738.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: MEDL; L; Abridged Index Medicus Journals; Priority Journals; Cancer Journals
LANGUAGE: English
OTHER SOURCE: MEDLINE 96420598
ENTRY MONTH: 199612

AB To characterize genes that become upregulated with malignant transformation of human hepatocytes, a library of monoclonal antibodies was produced against the FOCUS hepatocellular carcinoma cell line. Antibody FB-50 reacted with an antigen that was highly expressed in 4 of 10 primary hepatocellular carcinomas, in all 20 cholangiocarcinomas we studied, and in a variety of transformed cell lines. This antigen was also highly expressed in neoplastic epithelial cells of breast and colon carcinomas in contrast to its low level of expression in normal hepatocytes and in non-neoplastic epithelial cells. Among the normal adult tissues studied, high levels were observed only in proliferating trophoblastic cells of the placenta and in adrenal glands. A 636-bp partial cDNA, isolated from a gamma GT11 expression library generated with HepG2 human hepatoblastoma cells, and a complete cDNA, generated by reverse transcriptase-PCR, identified the antigen as the human form of **aspartyl(asparaginyl)beta-hydroxylase**. This enzyme catalyzes posttranslational hydroxylation of **beta** carbons of specific **aspartyl** and **asparaginyl** residues in EGF-like domains of certain proteins. Analyses of extracts prepared from several human tumor cell lines compared to their normal tissue counterparts indicate that the increase in **hydroxylase**, approximately 10-fold, is controlled at the level of transcription and the protein is expressed in an enzymatically active form. In similar analyses, comparing hepatocellular carcinomas to adjacent uninvolved liver from five patients, enzymatic activity was much higher in the tumor tissue from the four patients whose immunoblots revealed increased **hydroxylase** protein in the malignant tissue. EGF repeats in the extracellular domain of Notch or its homologs contain the consensus sequence for hydroxylation. Deletion mutants lacking this domain are gain-of-function mutants, suggesting that the domain modulates signal transduction by the cytoplasmic domain. While the function imparted by **beta** hydroxylation is unknown, our studies raise the possibility that **beta** hydroxylation is regulated in proteins like the mammalian Notch homologs, whose cytoplasmic domains have been shown to be oncogenic.

L23 ANSWER 3 OF 3 CANCERLIT
ACCESSION NUMBER: 95121937 CANCERLIT
DOCUMENT NUMBER: 95121937
TITLE: Cloning and characterization of the human gene

encoding **aspartyl beta-hydroxylase**.

AUTHOR: Korioth F; Gieffers C; Frey J
CORPORATE SOURCE: Universitat Bielefeld, Fakultat fur Chemie, Germany.
SOURCE: GENE, (1994). Vol. 150, No. 2, pp. 395-9.
Journal code: FOP. ISSN: 0378-1119.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: MEDL; L; Priority Journals
LANGUAGE: English
OTHER SOURCE: MEDLINE 95121937
ENTRY MONTH: 199503

AB Sequence information for **aspartyl beta-hydroxylase** (AspH), which specifically hydroxylates one Asp or Asn residue in certain epidermal growth factor (EGF)-like domains of a number of proteins, is so far only described for bovine species. We have isolated a 4.3-kb cDNA encoding the human AspH (hAspH) by immunoscreening of a human osteosarcoma (MG63) cDNA library in lambda ZAP with an antiserum raised against membrane fractions of these cells. Northern blot analyses revealed two transcripts with lengths of 2.6 and 4.3 kb. The deduced amino acid (aa) sequence of this cDNA encodes a protein of 757 aa (85 kDa). Comparison with the deduced bovine AspH (bAspH) aa sequence showed striking differences in the N-terminal portion of this protein. In vitro transcription and translation in the presence of canine pancreas microsomes yielded a 56-kDa protein. Western blot analyses of membrane fractions from MG63 cells with AspH-specific antibodies revealed a protein of the same M(r). These results suggest a posttranslational cleavage of the catalytic C terminus in the lumen of the endoplasmic reticulum.

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, USPATFULL, CANCERLIT' ENTERED AT 15:32:51 ON 22 OCT 1999)

- Author(s)

L24 601 S RADOSEVICH J?/AU
L25 366 S L24 AND (CANCER? OR CARCIN? OR TUMOUR OR TUMOR OR NEOPLAS?)
L26 64 S L25 AND MARKER
L27 29 DUP REM L26 (35 DUPLICATES REMOVED)

Searcher : Shears 308-4994

L27 ANSWER 1 OF 29 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:614146 CAPLUS
TITLE: cloning, cDNA sequence and mapping of a human gene encoding labyrinthin, a **cancer** -associated antigen
INVENTOR(S): **Radosevich, James A.**
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9947683	A1	19990923	WO 1999-US5365	19990311

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1998-40485 19980317

AB A cDNA mol. that encodes a protein designated Labyrinthin (Lab), the adenocarcinoma-assocd. antigen recognized by the monoclonal antibody (MCA) 44-3A6, is isolated and its nucleotide sequence is detd. Labyrinthin has about a 99.6% homol. with an internal segment of the protein coding region for human aspartyl .beta.-hydrolase (HAAH). The protein, or peptides derived from the protein, are **markers** useful to define novel classes of **cancers**. Diagnostic assays for these **cancers** use antibodies to Lab or nucleotide probes that hybridize with the lab gene or a fragment therefrom. Vaccines useful either to prevent recurrence of **cancers** in subjects who test pos. for Lab (or lab), or to prevent initial occurrence of **cancer**, use proteins or peptides derived from Lab. Expression of Lab via immunogenic assays is used to monitor effects of **cancer** treatments. Antisense mols. against lab are used in treatments. Sense mols. of lab are used to restore lost lab function in diseased normal cells, for example, gland cells.

L27 ANSWER 2 OF 29 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1998252554 MEDLINE
DOCUMENT NUMBER: 98252554
TITLE: Expression of the adenocarcinoma-related antigen

recognized by monoclonal antibody 44-3A6 in salivary gland **neoplasias**.

AUTHOR: Bentz B G; Haines G K 3rd; vonSchlegell A S; Elseth K M; Hanson D G; **Radosevich J A**

CORPORATE SOURCE: Department of Otolaryngology-Head and Neck Surgery, Robert H. Lurie Cancer Center, VA Lakeside Medical Center, and Northwestern University Medical School, Chicago, Illinois, USA.

CONTRACT NUMBER: T32-DC00015-13 (NIDCD)

SOURCE: OTOLARYNGOLOGY - HEAD AND NECK SURGERY, (1998 May) 118 (5) 603-9.
Journal code: ON8. ISSN: 0194-5998.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199808

ENTRY WEEK: 19980801

AB The monoclonal antibody 44-3A6 detects a cell-surface protein that has been shown to be a useful **marker** in distinguishing adenocarcinomas from other histologic **tumor** types in a variety of tissues. The objective of this study was to determine whether 44-3A6 could be used as a tool in the classification of salivary gland **neoplasms**. These complex **tumors** share overlapping pathologic features but distinct clinical outcomes. This study used 44-3A6 to immunohistochemically describe the pattern and frequency of this antigen in salivary gland **neoplasms**. Formalin-fixed, paraffin-embedded tissue sections of 22 benign and 26 malignant salivary **tumors** were evaluated. The patient population consisted of 25 (52.1%) women and 23 (47.9%) men selected from archival pathology files to reflect a range of salivary gland diseases. Normal surrounding salivary glands were found to have intense focal staining almost exclusively localized to ductal luminal cells. There was little staining of either myoepithelial or acinar cells. A wide spectrum of expression was found between and within **tumor** types, but a trend toward more expression of this antigen with decreasing differentiation was seen. A significant increase in staining was also seen in those **tumors** with ductal differentiation (n = 41) as opposed to those with predominantly acinar (i.e., acinic cell **carcinoma**) or myoepithelial (i.e., myoepithelioma; n = 8) differentiation (2.6 vs. 1.3, $p < 0.05$). No correlation was found between staining intensity and facial paralysis, pain, skin involvement, TNM stage, residual disease, or disease-free or total survival. Therefore this antigen appears to designate a duct luminal phenotype in normal and **neoplastic** salivary tissues.

L27 ANSWER 3 OF 29 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 1998084000 MEDLINE

Searcher : Shears 308-4994

DOCUMENT NUMBER: 98084000
TITLE: Interferon-responsive protein kinase (p68) and proliferating cell nuclear antigen are inversely distributed in head and neck squamous cell **carcinoma**.
AUTHOR: Haines G K 3rd; Panos R J; Bak P M; Brown T; Zielinski M; Leyland J; **Radosevich J A**
CORPORATE SOURCE: Department of Pathology, VA Lakeside Medical Center/Northwestern University Medical School, Chicago, Ill. 60611, USA.
SOURCE: TUMOUR BIOLOGY, (1998) 19 (1) 52-9.
Journal code: TUB. ISSN: 1010-4283.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199803
ENTRY WEEK: 19980304

AB PKR (protein kinase, interferon-responsive) is a ribosomal-associated protein kinase found in all human cells. When activated by dsRNA or polyanionic substances, PKR efficiently inhibits cellular protein synthesis. PKR expression has been correlated with cellular differentiation in a number of **tumor** types, including squamous cell **carcinoma** of the head and neck region. Although transfection of PKR into mouse fibroblasts and yeast cells inhibits proliferation, it is not known if modulation of native PKR levels occurs during cellular proliferation and differentiation in human normal and **neoplastic** tissues. To determine whether PKR expression was inversely related to proliferative activity in vivo, we used double-label immunohistochemistry to colocalize PKR and the proliferation **marker**, proliferating cell nuclear antigen (PCNA), in a series of head and neck squamous cell **carcinomas**. Overall, **neoplasms** demonstrating high levels of PKR showed low levels of PCNA immunoreactivity; **carcinomas** with low levels of PKR expressed high levels of PCNA. Within individual **tumors**, PKR and PCNA showed an inverse regional distribution: PKR was located predominantly in the center of **tumor** nests, while PCNA was restricted to the periphery. Patients whose **tumors** expressed high levels of both PKR and PCNA had the longest mean disease-free survival. These findings support the hypothesis that PKR levels are modulated in cell proliferation and differentiation in head and neck squamous cell **carcinoma**. Further studies are needed to clarify the mechanisms underlying the antiproliferative activity of PKR.

L27 ANSWER 4 OF 29 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998017649 EMBASE
TITLE: Interferon-responsive protein kinase (p68) and

proliferating cell nuclear antigen are inversely distributed in head and neck squamous cell **carcinoma**.

AUTHOR: Haines III G.K.; Panos R.J.; Bak P.M.; Brown T.; Zielinski M.; Leyland J.; **Radosevich J.A.**

CORPORATE SOURCE: Dr. G.K. Haines III, Department of Pathology, Northwestern Memorial Hospital, 3031 Superior St., Chicago, IL 60611, United States

SOURCE: Tumor Biology, (1997) 19/1 (52-59).
Refs: 33
ISSN: 1010-4283 CODEN: TUMBEA

COUNTRY: Switzerland

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 005 General Pathology and Pathological Anatomy

ity of **tumors** with increased cellular differentiation, and those tissues with decreased p68 were, in general, less differentiated. These data are consistent with the concept that the expression of p68 parallels the degree of cellular differentiation, and are consistent with previously reported studies using this antibody. The limited fetal expression pattern of p68 in the colon and the variable correlation of p68 with differentiation suggests that p68, as well as other translational regulators, can be important in assessing the biological potential of **tumors** arising in the colon.

L27 ANSWER 6 OF 29 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:316780 CAPLUS

DOCUMENT NUMBER: 125:31069

TITLE: The double-stranded RNA-dependent protein kinase (p68) is a novel **marker** for differentiation in aero-digestive **tumors**

AUTHOR(S): Haines, G., III; **Radosevich, J.**

CORPORATE SOURCE: Medical School, Northwestern University, Chicago, IL, USA

SOURCE: Proc. Int. Cancer Congr., Free Pap. Posters, 16th (1994), Volume 3, 1683-1686. Editor(s): Rao, R. S. Monduzzi Editore: Bologna, Italy.
CODEN: 62UYAO

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The dsRNA-dependent protein kinase (P68) is noted for its marked anti-viral activity. The authors report that p68 may be a key regulator of differentiation in the aerodigestive tract. P68 expression increases with cellular maturation in squamous mucosa, and correlates with grade in squamous **carcinomas** from both the lung and head & neck region. P68 shows a regional gradient within **tumor** nests: low levels at the periphery (immature, actively dividing cells), higher levels in the more mature central zones. P68 may be a key factor in regulating differentiation.

Searcher : Shears 308-4994

L27 ANSWER 7 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1994:514770 BIOSIS
DOCUMENT NUMBER: PREV199497527770
TITLE: PH modulates the expression of labyrinthin: The human adenocarcinoma **marker** detected by the monoclonal antibody 44-3A6.
AUTHOR(S): **Radosevich, J. A. (1)**; Elseth, K. E.; Bradley, G. S.; Haines, G. K.
CORPORATE SOURCE: (1) Northwestern Univ., Chicago, IL USA
SOURCE: Clinical Research, (1994) Vol. 42, No. 3, pp. 404A. Meeting Info.: Combined Annual Meeting of the Central Society for Clinical Research, American Federation for Clinical Research, Midwest Section, Midwest Society for Pediatric Research, Society for Investigative Dermatology, Central Region, and the Midwest Society of General Internal Medicine Chicago, Illinois, USA September 16-18, 1994
ISSN: 0009-9279.
DOCUMENT TYPE: Conference
LANGUAGE: English

L27 ANSWER 8 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 4
ACCESSION NUMBER: 1993:64397 BIOSIS
DOCUMENT NUMBER: PREV199344030047
TITLE: Monoclonal antibody TJ4C4 as a **marker** for breast **neoplasms**.
AUTHOR(S): Cajulis, R. S. (1); Hayden, R.; Hidvegi, D. F.; Duda, R. B.; August, C. Z.; **Radosevich, J. A.**
CORPORATE SOURCE: (1) Dep. Pathol., Northwestern Univ./V.A. Lakeside Med. Cent., Chicago, Ill
SOURCE: Clinical Research, (1992) Vol. 40, No. 3, pp. 754A. Meeting Info.: Meeting of the Midwest Section American Federation for Clinical Research Chicago, Illinois, USA November 4-6, 1992
ISSN: 0009-9279.
DOCUMENT TYPE: Conference
LANGUAGE: English

L27 ANSWER 9 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1993:64398 BIOSIS
DOCUMENT NUMBER: PREV199344030048
TITLE: Human fetal expression of the protein kinase (P68) using the monoclonal antibody TJ4C4.
AUTHOR(S): Haines, G. K. (1); Ghadge, G.; **Radosevich, J. A.**
CORPORATE SOURCE: (1) Dep. Pathol., Northwestern Univ./V.A. Lakeside Med. Cent., Chicago, Ill
SOURCE: Clinical Research, (1992) Vol. 40, No. 3, pp. 754A.

Meeting Info.: Meeting of the Midwest Section American Federation for Clinical Research Chicago, Illinois, USA November 4-6, 1992
ISSN: 0009-9279.
DOCUMENT TYPE: Conference
LANGUAGE: English

L27 ANSWER 10 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1992:442093 BIOSIS
DOCUMENT NUMBER: BR43:75093
TITLE: IMMUNOHISTOCHEMICAL ANALYSIS OF DIFFERENTIATION **MARKERS** FOR NON-SMALL-CELL LUNG **CANCER**.
AUTHOR(S): **RADOSEVICH J A**; ROSEN S T; COMBS S G
CORPORATE SOURCE: NORTHWESTERN UNIV., V.A. LAKESIDE MED. CENT., CHICAGO, ILL.
SOURCE: BERNAL, S. D. AND P. J. HESKETH (ED.). LUNG BIOLOGY IN HEALTH AND DISEASE, VOL. 58. LUNG CANCER DIFFERENTIATION: IMPLICATIONS FOR DIAGNOSIS AND TREATMENT. XXVI+488P. MARCEL DEKKER, INC.: NEW YORK, NEW YORK, USA; BASEL, SWITZERLAND. ILLUS, (1992) 0 (0), 195-216.
CODEN: LBHDD7. ISSN: 0362-3181. ISBN: 0-8247-8638-6.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L27 ANSWER 11 OF 29 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 5
ACCESSION NUMBER: 1993:99168 CAPLUS
DOCUMENT NUMBER: 118:99168
TITLE: Expression of the protein kinase p-68 recognized by the monoclonal antibody TJ4C4 in human lung **neoplasms**
AUTHOR(S): Haines, G. K.; Ghadge, G.; Thimmappaya, B.; **Radosevich, J. A.**
CORPORATE SOURCE: Dep. Pathol., Northwestern Univ., Chicago, IL, 60611, USA
SOURCE: Virchows Arch., B (1992), 62(3), 151-8
CODEN: VAAZA2; ISSN: 0340-6075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB P68 is a protein kinase expressed by eukaryotic cells, which is inducible by alpha interferon, and is believed to be an important factor in the regulation of viral and cellular protein synthesis. The authors have previously reported on a monoclonal antibody, TJ4C4, which is able to specifically detect p68 in formalin-fixed, paraffin-embedded tissue. Because of its important role in regulating cellular protein synthesis, the authors hypothesized that p68 expression would vary among lung **neoplasms** with the level of differentiation and degree of biosynthetic activity. A

total of 246 untreated primary pulmonary and pleural **neoplasms** were studied. The frequency and relative intensity of p68 expression was detd. by light microscopic evaluation of ABC immunoperoxidase stained specimens. All categories of **tumors** studied demonstrated a spectrum of p68 expression. Expression of p68 correlated well with degree of differentiation in squamous cell **carcinomas** (SQCC) and acinar adenocarcinomas (AAC). Papillary adenocarcinoma (PAC) and bronchoalveolar **carcinoma** (BAC) expressed low levels of p68, despite their well differentiated appearance. Expression of the antigen in large cell **carcinoma** (LCC) was higher than that seen in either poorly differentiated AAC or SQCC. Neuroendocrine **tumors** generally showed low levels of p68 expression with the intermediate variant of small cell **carcinoma** expressing higher levels of p68 than the classic "oat cell" form (SCC). **Carcinoid tumors** expressed higher levels of p68 than did atypical **carcinoid tumors**. Mesotheliomas showed weak expression of p68, limited primarily to areas of glandular differentiation in the epithelioid form. Differential expression of p68 by lung and pleural **neoplasms** points to biol. differences among these **tumors**, serves as a good biol. **marker** for cellular differentiation, and may provide clin. relevant information about individual **tumor** subtypes.

L27 ANSWER 12 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1992:442087 BIOSIS
DOCUMENT NUMBER: BR43:75087
TITLE: A CELLULAR 68000 MW PROTEIN AS A DIFFERENTIATION **MARKER** IN HUMAN LUNG **CANCER**.
AUTHOR(S): **RADOSEVICH J A**; HAINES G K; GHADGE G; THIMMAPAYA B
CORPORATE SOURCE: NORTHWESTERN UNIV., V.A. LAKESIDE MED. CENT., CHICAGO, ILL.
SOURCE: BERNAL, S. D. AND P. J. HESKETH (ED.). LUNG BIOLOGY IN HEALTH AND DISEASE, VOL. 58. LUNG CANCER DIFFERENTIATION: IMPLICATIONS FOR DIAGNOSIS AND TREATMENT. XXVI+488P. MARCEL DEKKER, INC.: NEW YORK, NEW YORK, USA; BASEL, SWITZERLAND. ILLUS, (1992) 0 (0), 87-102.
CODEN: LBHDD7. ISSN: 0362-3181. ISBN: 0-8247-8638-6.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L27 ANSWER 13 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1991:329362 BIOSIS
DOCUMENT NUMBER: BR41:25912
TITLE: **TUMOR MARKERS** AND PHENOTYPING TECHNIQUES.

AUTHOR(S): COHN S L; ROBINSON P G; **RADOSEVICH J A**; ROSEN S T
CORPORATE SOURCE: DIV. HEMATOL./ONCOL., CHILDREN'S MEML. HOSP., CHICAGO, ILL.
SOURCE: ECONOMOU, S. G., T. R. WITT, D. J. DEZIEL, T. J. SACLARIDES, E. D. STAREN AND S. D. BINES. ADJUNCTS TO CANCER SURGERY. XXV+703P. LEA AND FEBIGER: PHILADELPHIA, PENNSYLVANIA, USA; LONDON, ENGLAND, UK. ILLUS, (1991) 0 (0), 207-218.
ISBN: 0-8121-1327-6.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L27 ANSWER 14 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1991:191761 BIOSIS
DOCUMENT NUMBER: BR40:89041
TITLE: MEDIASTINAL **TUMOR MARKERS**.
AUTHOR(S): ROBINSON P G; **RADOSEVICH J A**
CORPORATE SOURCE: UNIV. MIAMI SCH. MED., MIAMI, FLA.
SOURCE: SHIELDS, T. W. (ED.). MEDIASTINAL SURGERY. X+400P. LEA AND FEBIGER: MALVERN, PENNSYLVANIA, USA; LONDON, ENGLAND, UK. ILLUS, (1991) 0 (0), 62-75.
ISBN: 0-8121-1362-4.
FILE SEGMENT: BR; OLD
LANGUAGE: English

L27 ANSWER 15 OF 29 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 92073853 MEDLINE
DOCUMENT NUMBER: 92073853
TITLE: Monoclonal antibody 44-3A6 as a **marker** for breast **carcinoma**.
AUTHOR: Duda R B; August C Z; Rosen S T; **Radosevich J A**
CORPORATE SOURCE: Department of Surgery, Northwestern University Medical School, Chicago, Ill..
SOURCE: TUMOUR BIOLOGY, (1991) 12 (5) 254-60.
Journal code: TUB. ISSN: 1010-4283.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199203
AB The monoclonal antibody (MAb) 44-3A6 detects a 40-kD cell surface protein on adenocarcinomas and may serve as an effective **marker** for glandular differentiation. Immunohistochemical analysis of 123 paraffin-embedded malignant breast tissue specimens, 27 normal or benign breast disease specimens and 10 atypical hyperplasia specimens from patients without breast **cancer** was performed with MAb 44-3A6. The antigen was identified in 76% of

breast **cancer** specimens, 0% of normal or benign breast disease specimens and 88% of the atypical hyperplasia specimens. MAb 44-3A6 also detected this antigen on adjacent normal breast ductal cells in 88% of the breast **cancer** specimens. There was no statistically significant correlation between immunoreactivity and histological mitotic or nuclear grade, recurrence or overall survival. This study suggests that the cell surface antigen detected by the MAb 44-3A6 may serve as an important **marker** in the differentiation of normal breast epithelium into an atypical or malignant lesion.

L27 ANSWER 16 OF 29 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 90296581 MEDLINE
DOCUMENT NUMBER: 90296581
TITLE: Immunohistochemical analysis of pulmonary and pleural **neoplasms** with monoclonal antibodies B72.3 and CSLEX-1.
AUTHOR: Koukoulis G K; **Radosevich J A**; Warren W H; Rosen S T; Gould V E
CORPORATE SOURCE: Department of Pathology, Rush Medical College, Chicago, IL 60612..
SOURCE: VIRCHOWS ARCHIV. B, CELL PATHOLOGY INCLUDING MOLECULAR PATHOLOGY, (1990) 58 (6) 427-33. Journal code: BWO. ISSN: 0340-6075.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199010
AB Sequential paraffin sections of 222 epithelial lung **tumors** comprising all common histologic types, and 31 pleural mesotheliomas of all variants were immunostained with monoclonal antibodies (Mabs) B72.3 and CSLEX-1. Reactivity with Mabs B72.3 and CSLEX-1 respectively was noted in 7/57 and 4/57 squamous **carcinomas**, in 44/70 and 60/70 adenocarcinomas, 9/16 and 11/16 bronchioloalveolar **carcinomas**, 8/25 and 14/25 large cell undifferentiated **carcinomas**, 3/3 and 3/3 adenosquamous **carcinomas**, 0/11 and 0/11 **carcinoids**, 0/10 and 2/10 well differentiated neuroendocrine (NE) **carcinomas**, 4/13 and 5/13 intermediate cell NE **carcinomas**, 0/17 and 0/17 small cell NE **carcinomas**, and 0/31 and 1/31 mesotheliomas. In most instances, both Mabs stained the same **tumors**; however, reactivity with CSLEX-1 was more intense and extensive, and involved more cases. Therefore, regardless of conventional histologic type, staining with Mabs B72.3 and CSLEX-1 defines 4 subsets of lung **tumors**: one expressing both antigens, two expressing one but not the other, and one expressing neither. The possible biological and/or clinical significance of these subsets remains undetermined. When correlated with

conventional histologic **tumor** types, our findings indicate: 1). both of these Mabs recognize most but not all adenocarcinomas and bronchioloalveolar **carcinomas**, and since CSLEX-1 stained more cases than B72.3, it may be argued that the former is a broader exocrine phenotype **marker** than the latter; 2). both of these Mabs select exocrine subsets of large cell undifferentiated **carcinomas**; 3). both of these Mabs stain exocrine cell subpopulations in well differentiated and intermediate cell NE **carcinomas** but not in **carcinoids** or small cell NE **carcinomas**, and 4). except for rare cases, neither B72.3 nor CSLEX-1 reacts with mesotheliomas regardless of variant.(ABSTRACT TRUNCATED AT 250 WORDS)

L27 ANSWER 17 OF 29 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 90298149 EMBASE
DOCUMENT NUMBER: 1990298149
TITLE: Immunohistochemical analysis of human adenocarcinomas of the lung using the monoclonal antibody 44-3A6.
AUTHOR: **Radosevich J.A.**; Noguchi M.; Rosen S.T.; Shimosato Y.
CORPORATE SOURCE: Division of Pathology, National Cancer Center Research Institute, Tokyo, Japan
SOURCE: Tumor Biology, (1990) 11/4 (181-188).
ISSN: 1010-4283 CODEN: TUMBEA
COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
015 Chest Diseases, Thoracic Surgery and Tuberculosis
016 Cancer
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In this study, 39 primary, surgically resected, pulmonary adenocarcinomas of hhe following cell types were investigated: 19 Clara cell, 4 bronchial-gland, 15 goblet cell, and 1 type II alveolar epithelial adenocarcinoma. They were analyzed for the expression of the glandular differentiation-associated antigen recognized by the monoclonal antibody 44-3A6. Formalin-fixed, paraffin-embedded tissue sections were immunostained using the avidinbiotin complex/peroxidase method. All of the Clara cell **tumors** (19/19) expressed this antigen as well as 3/4 bronchial-gland **tumors**, as compared to only 2/15 goblet **tumors**. The single type II pneumocyte **neoplasm** studied was found to express this antigen. These findings parallel our earlier observations based on cytological features, and provide supportive evidence that there may be biological differences between human pulmonary adenocarcinomas and bronchioloaveolar **carcinomas**.

Searcher : Shears 308-4994

L27 ANSWER 18 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1990:111389 BIOSIS
DOCUMENT NUMBER: BA89:60880
TITLE: IMMUNOHISTOCHEMICAL ANALYSIS OF PULMONARY AND PLEURAL **NEOPLASMS** USING A MONOCLONAL ANTIBODY 47D10 WHICH REACTS WITH NONSPECIFIC CROSS-REACTING ANTIGEN.
AUTHOR(S): **RADOSEVICH J A**; ROBINSON P G; CARNEY W P; WARREN W; ROSEN S T; GOULD V E
CORPORATE SOURCE: NORTHWESTERN UNIVERSITY, SECTION HEMATOLOGY/ONCOLOGY, OLSON 8340, 303 E. CHICAGO AVENUE, CHICAGO, IL 60611, USA.
SOURCE: TUMOR BIOL, (1989) 10 (6), 281-288.
CODEN: TUMBEA.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A series of 251 human pulmonary **carcinomas** were analyzed immunohistochemically for the antigens recognized by a new monoclonal antibody (MAb) 47D10. These antigens are part of a complex family of substances similar to, yet distinct from **carcinoembryonic** antigen (CEA), and are termed 'nonspecific cross-reacting antigens' (NCAs). The NCA epitope recognized by the MAb 47D10 is expressed on the cell surface and has previously been shown to be distinct from epitopes detected by several anti-CEA MAbs, as well as by MAbs 19-9 and Du-PAN-2. The NCA epitope recognized by MAb 47D10 is well preserved in formalin-fixed and paraffin-embedded tissues. Uisng immunohistochemistry, this epitope has been shown to have a limited biodistribution in normal tissues, and to be expressed by adenocarcinomas arising in the pancreas, colon, breast, ovary, prostate and lung. The frequency and pattern of NCA expression in human pulmonary **neoplasma** was found to correlate with the known distribution of CEA; and was often present in the non-small-cell **carcinomas**. In addition, the expression of CEA relative to NCA was evaluated in a select group of non-small-cell **carcinoma** cases using several anti-CEA MAbs, to directly compare the expression of CEA to NCA. In general, the NCA reaction pattern is more intense and expressed on more cells within the **tumors** than that of CEA expression.

L27 ANSWER 19 OF 29 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 90048706 MEDLINE
DOCUMENT NUMBER: 90048706
TITLE: Immunohistochemical analysis of pulmonary and pleural **tumors** with the monoclonal antibody HYB-612 directed against the multidrug resistance (MDR-1) gene product, P-glycoprotein.
AUTHOR: **Radosevich J A**; Robinson P G; Rittmann-Grauer L S; Wilson B; Leung J P; Maminta M L; Warren W; Rosen S T; Gould V E
CORPORATE SOURCE: Department of Pathology, Northwestern

University/Veterans Administration Lakeside Medical Center, Chicago, Ill..
SOURCE: TUMOUR BIOLOGY, (1989) 10 (5) 252-7.
Journal code: TUB. ISSN: 1010-4283.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199002
AB In this study, 212 untreated primary pulmonary and pleural **neoplasms** were studied immunohistochemically with the monoclonal antibody HYB-612 which detects the multidrug resistance (MDR)-related P-glycoprotein (gp180). A **tumor** was considered positive for the expression of the MDR phenotype, even if a single rare positive cell was detected. Using this criterion, all of the various histologic subtypes were found to express MDR to varying degrees. The frequency of expression of this phenotype was found to be notably higher in non-small-cell **carcinomas** than in small-cell **carcinomas**. These findings are consistent with the known clinical responses of these **neoplasms**. The detection of gp180 in untreated lung **neoplasms** may be predictive of the responsiveness of **neoplasms** to chemotherapeutic agents. In addition, its presence or absence might be useful in determining the appropriate treatment protocol for given patients.

L27 ANSWER 20 OF 29 CANCERLIT
ACCESSION NUMBER: 89650573 CANCERLIT
DOCUMENT NUMBER: 89650573
TITLE: BIOLOGY OF LUNG **CANCER**.
AUTHOR: Rosen S T; **Radosevich J A**
CORPORATE SOURCE: Section of Hematology/Oncology, Dept. of Medicine, Northwestern Univ., Chicago, IL.
SOURCE: Non-serial, (1988). Lung Cancer:A Comprehensive Treatise. Bitran JD et al, eds. Orlando, FL, Grune and Stratton.
DOCUMENT TYPE: Book; (MONOGRAPH)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
FILE SEGMENT: ICDB; L
LANGUAGE: English
ENTRY MONTH: 198904
AB The major histologic types of lung **cancer** include squamous cell, adenocarcinoma, large cell **carcinoma**, and small cell **carcinoma** (SCLC). The first three histologies are collectively referred to as non-small cell lung **carcinomas** (NSCLC). They are distinguished from SCLC by clinical presentation, response to chemotherapy and radiation therapy, and biologic characteristics. Dramatic advances in knowledge of the basic

cellular and molecular biology of lung **cancer** have occurred in the last decade. The biology of lung **cancer** is discussed. Topics include the development of human lung **cancer** cell lines; cell line characteristics; in vitro soft agarose cloning; flow cytometric DNA content analysis; cytogenetics; oncogenes; peptide hormone production, growth factors, and receptors; enzyme **markers**; cytoskeletal proteins; and monoclonal antibodies against lung **cancer**-associated antigens. The establishment of SCLC and NSCLC lines has facilitated detailed biologic investigation of lung **cancer**. The similarities and differences among these **tumor** types, observed in vitro, have also been observed in clinical specimens. In addition, within each lung **cancer** cell type, morphologic, biochemical, and molecular heterogeneity is apparent. These cell lines have contributed to our knowledge of the relationships among the various histologic subtypes of human lung **cancer**. (188 Refs)

L27 ANSWER 21 OF 29 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 89128568 MEDLINE
DOCUMENT NUMBER: 89128568
TITLE: Immunohistochemical identification of exocrine and neuroendocrine subsets of large cell lung **carcinomas**.
AUTHOR: Piehl M R; Gould V E; Warren W H; Lee I; **Radosevich J A**; Ma Y X; Rosen S T
CORPORATE SOURCE: Rush-Presbyterian-St. Luke's Medical Center, Department of Pathology, Chicago..
SOURCE: PATHOLOGY, RESEARCH AND PRACTICE, (1988 Nov) 183 (6) 675-82.
Journal code: PBZ. ISSN: 0344-0338.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198905
AB Formalin fixed, paraffin embedded sections of 52 cases of pulmonary large cell undifferentiated **carcinoma** (LCUC) as defined in the current WHO classification were studied immunohistochemically to assess features of exocrine and neuroendocrine (NE) differentiation. Monoclonal antibody 44-3A6 was applied to detect a membrane association protein related to exocrine differentiation. A panel of ten neuroendocrine **markers** including antibodies to synaptophysin, chromogranin A, serotonin, and seven neuropeptides was used to assess NE differentiation. The broad spectrum anticytokeratin antibody PKK1 was used to confirm the epithelial differentiation of these **tumors**. Exocrine differentiation was detected in 40/52 (77%) of surgically resected LCUC, despite the absence of recognizable glands by light microscopy. Eighteen of 52

(35%) LCUC exhibited NE differentiation; synaptophysin was the most frequently detected NE **marker**. Cytokeratin immunostaining with PKK1 was demonstrated in 41/52 (79%) cases. Subsets of LCUC were defined based on their expression of exocrine or NE phenotypic **markers**. Accordingly, 28/52 (54%) LCUC displayed an exocrine phenotype, 6/52 (12%) a NE phenotype, 12/52 (23%) had combined exocrine and NE phenotypes, and 6/52 (12%) exhibited neither phenotype. In this surgical series, there were no significant differences in stage at presentation for the four subsets. Interestingly, two year survival appeared decreased in patients with **tumors** displaying the "pure" NE phenotype.

L27 ANSWER 22 OF 29 CANCERLIT
ACCESSION NUMBER: 89657788 CANCERLIT
DOCUMENT NUMBER: 89657788
TITLE: NON-SMALL CELL LUNG **CANCER** ANTIGENS.
AUTHOR: **Radosevich J A**; Ma X; Weitzman S A; Rosen S T
CORPORATE SOURCE: Northwestern Univ./VA Lakeside Medical Center, Chicago, IL.
SOURCE: Lung Biol Health Dis, (1988). Vol. 37, pp. 237-53.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
FILE SEGMENT: ICDB; L
LANGUAGE: English
ENTRY MONTH: 198911
AB In order to help classify subtypes of human **tumors**, a panel has been established of over 200 primary lung **neoplasm** specimens, including squamous cell **carcinomas**; adenocarcinomas; large cell and bronchioalveolar **carcinomas**; small cell, intermediate small cell, and well-differentiated neuroendocrine **carcinomas**; and **carcinoids**. In addition, more than 60 mesotheliomas have been used in evaluation of monoclonal antibodies of (MoAbs) in the authors' laboratory. The use of this panel and of several MoAbs is described. Human non-small cell lung **carcinomas** are a heterogeneous group of **tumors**. Unless a **tumor** exhibits the classic features of a particular subtype, it can be difficult to classify using traditional light and electron microscopy. Great effort has been made since the advent of hybridoma technology to produce reagents to resolve this problem. These MoAbs are beginning to define select subsets of **tumors**, and eventually a panel of these reagents will be available for routinely phenotyping pulmonary **carcinomas**. MoAbs not only will aid in the classification of these **tumors**, but also will be useful in understanding the biology of this disease. (46 Refs)

L27 ANSWER 23 OF 29 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 88176552 EMBASE
DOCUMENT NUMBER: 1988176552
TITLE: Expression of the antigenic determinant recognized by the monoclonal antibody 44-3A6 on select human adenocarcinomas and normal human tissues.
AUTHOR: Combs S.G.; **Radosevich J.A.**; Ma Y.; Lee I.; Gould V.E.; Battifora H.; Rosen S.T.
CORPORATE SOURCE: MacNeal Memorial Hospital, Department of Pathology, Berwan, IL, United States
SOURCE: Tumor Biology, (1988) 9/2-3 (116-122).
ISSN: 1010-4283 CODEN: TUMBEA
COUNTRY: Switzerland
DOCUMENT TYPE: Journal
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
016 Cancer
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The IgG1 monoclonal antibody, 44-3A6, was raised against the human lung adenocarcinoma cell line, A549. It has been shown to react with a 40,000 MW protein found on the cell surface, which is preserved in formalin-fixed paraffin-embedded tissues. A recent study of pulmonary **carcinomas** utilizing immunohistochemical methods showed exclusive binding to lung adenocarcinomas, subsets of neuroendocrine **tumors**, some **carcinoids** and a subset of large cell **carcinomas**. Reactivity was not seen in squamous cell **carcinomas** and small cell neuroendocrine **carcinomas**. In addition, melanomas, sarcomas and hematologic malignancies do not express the antigen. We now report on the reactivity pattern of 44-3A6 in adenocarcinomas of nonpulmonary primary sites and in normal adult organs. Strong diffuse staining of **neoplastic** cells in adenocarcinomas of the stomach, colon, pancreas, gallbladder and breast was noted. Adenocarcinomas arising in the endometrium, ovary, kidney, prostate, thyroid and liver were even noted in adenocarcinomas which had an 'undifferentiated' component; i.e., lacking well-defined glandular elements. Immunoreactivity was noted in epithelial cells in several tissues from which these adenocarcinomas arose including the bronchial tract, stomach, small intestine, pancreas and colon, whereas epithelial cells from the endometrium, kidney, ovary, prostate and thyroid were negative or showed diffuse weak immunoreactivity. Our findings indicate that monoclonal antibody 44-3A6 recognizes an epithelial antigen on subsets of normal as well as transformed glandular epithelia. The differential pattern of expression of its target antigen probably reflects differences in **tumor** genesis and/or differentiation.

L27 ANSWER 24 OF 29 CANCERLIT
ACCESSION NUMBER: 89650836 CANCERLIT
Searcher : Shears 308-4994

DOCUMENT NUMBER: 89650836
TITLE: MONOCLONAL ANTIBODY ASSAYS FOR LUNG **CANCER**.
AUTHOR: **Radosevich J A**; Rosen S T; Lee I; Gould V E
CORPORATE SOURCE: Northwestern Univ./VA Lakeside Medical Center, Chicago, IL.
SOURCE: Immunol Ser, (1988). Vol. 39, pp. 101-21.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
FILE SEGMENT: ICDB; L
LANGUAGE: English
ENTRY MONTH: 198904

AB The pathological classification of lung **cancer** is summarized, and the use of specific monoclonal antibodies (MAbs) in clarifying the ambiguity of traditional definitions is discussed. MAbs considered include 624A12; 44-3A6; KS 1/4 and K 1/17; LuCa2, LuCa3, and LuCa4; B72.3; LAM-8; SM1; and RAP-5 and DWP. Cytoskeletal **markers**, neuroendocrine **markers**, and serological assays for **carcinoembryonic** antigen, human chorionic gonadotropin, alpha-fetoprotein, calcitonin, neuron-specific enolase, creatine kinase BB, and sialosylated Lewis(x) and sialosylated Lewis(a) are also considered. The major problem in the design of new treatment strategies for lung **cancer** is rooted in the initial pathological diagnosis. Light and electron microscopic evaluations of lung **tumors** fail to allow consistent classification of these **tumors**, especially those of mixed cell types and those that lack salient features of any given **tumor** subtype. With the advent of MAb technology, the hope of overcoming this problem was raised. Over 200 MAbs have been reported to react with human lung **tumors**. Many of the reagents work only on frozen tissues and have not been extensively tested for their binding specificity to various subclasses of pulmonary **carcinomas**. Some of the MAbs are able to recognize their respective antigen in formalin-fixed, paraffin-embedded tissues. In an attempt to standardize and test the utility of some of these MAbs, a core bank of approx 200 human pulmonary **tumors** was created. Some of the MAbs described in this review were tested against this panel and the results are summarized. It is clear that these reagents will not replace conventional microscopic evaluation, but they may serve as useful adjuncts in defining the subclasses of lung **cancer**. (45 Refs)

L27 ANSWER 25 OF 29 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 10
ACCESSION NUMBER: 1987:405133 CAPLUS
DOCUMENT NUMBER: 107:5133
TITLE: Synaptophysin expression in neuroendocrine **neoplasms** as determined by immunocytochemistry
Searcher : Shears 308-4994

AUTHOR(S): Gould, Victor E.; Wiedenmann, Bertram; Lee, Inchul; Schwechheimer, Karl; Dockhorn-Dworniczak, Barbara; **Radosevich, James A.**; Moll, Roland; Franke, Werner W.
CORPORATE SOURCE: Dep. Pathol., Rush Med. Coll., Chicago, IL, 60612, USA
SOURCE: Am. J. Pathol. (1987), 126(2), 243-57
CODEN: AJPAA4; ISSN: 0002-9440
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Synaptophysin is an integral membrane glycoprotein originally isolated from presynaptic vesicle of bovine neurons. A wide spectrum of neuroendocrine (NE) **neoplasms** were studied by immunofluorescence microscopy on cryostat sections of freshly frozen tissues using a monoclonal antibody to this protein (SY 38). Without exception, the identical-or a very similar-protein was expressed in all neuroblastomas, ganglioneuroblastomas, ganglioneuromas, pheochromocytomas, and paragangliomas studied. In these neural type NE **neoplasms**, synaptophysin was coexpressed with neurofilament proteins. Synaptophysin was also demonstrated in NE **neoplasms** of epithelial type in which it was predominantly coexpressed with cytokeratins and desmoplakin. It was invariably found in all variants of islet cell **neoplasms** and in all medullary thyroid **carcinomas**. Synaptophysin was also demonstrated in several adenomas of the hypophysis and parathyroids, in the majority of **carcinoids** of the bronchopulmonary and gastrointestinal tracts, and in many not all, NE **carcinomas** of the same sites, of the skin. Conversely, SY 38 did not immunostain any of a large no. of benign and malignant non-NE epithelial **neoplasms**; nor was any immunostaining obtained in a group of mesenchymal **tumors**. It is remarkable that SY 38 did not immunostain a no. of malignant melanomas, including several that were immunostained for neuron-specific enolase (NSE) and several neuropeptides. Parallel studies conducted on conventionally fixed, paraffin-embedded tissue sections immunostained by the use of the avidin-biotin complex technique yielded very similar results. Thus, synaptophysin is expressed in the whole range of NE **neoplasms** without detectable relation to the expression of other NE **markers** such as NSE, serotonin, and neuropeptides. Nor could the expression of synaptophysin by these **tumors** be correlated with their epithelial and/or neural cytoskeletal characteristics, their clin. aggressiveness, or the presence or absence of endocrinol. abnormalities. Whereas the consistent expression of synaptophysin by the neural type of NE **neoplasms** would seem predictable, its presence in diverse benign and malignant NE **tumor** of epithelial type is remarkable. Thus, synaptophysin is a significant as well as novel NE **marker**, and the use of antibody SY 38 as a broad range **marker** for the study and diagnosis of NE

neoplasms is proposed.

L27 ANSWER 26 OF 29 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1987:422470 BIOSIS
DOCUMENT NUMBER: BA84:89132
TITLE: IMMUNOHISTOCHEMICAL EVALUATION OF RAS ONCOGENE EXPRESSION IN PULMONARY AND PLEURAL **NEOPLASMS**
AUTHOR(S): LEE I; GOULD V E; **RADOSEVICH J A**; THOR A; MA Y; SCHLOM J; ROSEN S T
CORPORATE SOURCE: RUSH-PRESBYTERIAN-ST. LUKE'S MED. CENT., DEP. PATHOL., 1753 W. CONGRESS PARKWAY, CHICAGO, IL 60612.
SOURCE: VIRCHOWS ARCH B CELL PATHOL INCL MOL PATHOL, (1987) 53 (3), 146-152.
CODEN: VABPDE.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB We undertook in immunohistochemical analysis of human bronchopulmonary epithelial **neoplasms** and pleural mesotheliomas using a monoclonal antibody which recognizes ras oncogene products (p21ras). The monoclonal antibody, RAP-5, recognizes both unaltered and certain mutated p21ras. Formation fixed and paraffin embedded tissue samples of 187 lung epithelial **tumors** and 27 pleural mesotheliomas were investigated; normal and bronchiectatic lungs were similarly studied. Normal lung and pleural tissue did not immunostain except for occasional type II pneumocytes. Reactive type II pneumocytes adjacent to **carcinomas** and bronchiectasis immunostained consistently. Twenty four/34 (71%) squamous **carcinomas** immunostained. Only 8/50 (16%) adneocarcinomas immunostained focally and weakly whereas 19/24 (79%) bronchioloalveolar **carcinomas** immunostained. Eleven/18 (61%) large cell **carcinomas** immunostained with variable intensity. Eleven/13 (85%) **carcinoids**, 6/7 (85%) well differentiated neuroendocrine carinomas, and 18/21 (86%) intermediate cell neuroendocrine **carcinomas** immunostained while none of 20 small cell neuroendocrine **carcinomas** immunostained. Only a few mesotheliomas were immunostained focally. Two/14 (14%) epithelial type and 1/9 (11%) biphasic type mesotheliomas immunostained weakly; none of 4 spindle cell mesotheliomas immunostained. We conclude that while at least occasional cases of most types of pulmonary epithelial **neoplasms** express p21ras, the frequency and intensity of the expression are distinctly greater in certain **tumor** types such as squamous, bronchioloalveolar, and neuroendocrine **neoplasm** except for the small cell type. Contrary to these lung epithelial **neoplasms**, most mesotheliomas did not immunostain for p21ras. Whether the enhanced p21ras expression may point to a different mechanism of transformation or may merely reflect differentiation features

remains undetermined.

L27 ANSWER 27 OF 29 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 86239543 MEDLINE
DOCUMENT NUMBER: 86239543
TITLE: Malignant mesotheliomas. Improved differential diagnosis from lung adenocarcinomas using monoclonal antibodies 44-3A6 and 624A12.
AUTHOR: Lee I; **Radosevich J A**; Chejfec G; Ma Y X; Warren W H; Rosen S T; Gould V E
SOURCE: AMERICAN JOURNAL OF PATHOLOGY, (1986 Jun) 123 (3) 497-507.
Journal code: 3RS. ISSN: 0002-9440.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals
ENTRY MONTH: 198609

AB Forty-three malignant pleural mesotheliomas and 10 known metastatic pulmonary adenocarcinomas to the pleura were studied by immunohistochemistry using monoclonal antibodies 44-3A6 and 624A12. Monoclonal antibodies 44-3A6 and 624A12 were raised against human pulmonary **carcinoma** cell lines; they recognize a membrane-associated protein of 40,000 mol wt and a specific sugar sequence of lacto-N-fucopentose III, respectively. Samples were also studied with a broad-spectrum antikeratin antibody and a polyclonal antibody to **carcinoembryonic** antigen (CEA). These investigations were performed on formalin-fixed and paraffin-embedded tissues. The mesotheliomas comprised only grossly evident, pleurectomized, or pneumonectomized cases; they included 22 epithelial, 15 biphasic, and 6 spindle cell types. Electron-microscopic study was also done on 9 cases. None of the mesotheliomas was immunoreactive to 624A12, while 9/10 metastatic pulmonary adenocarcinomas were convincingly immunoreactive. Monoclonal antibody 44-3A6 immunostained all of the metastatic adenocarcinomas strongly, whereas only 10/43 mesotheliomas were focally and weakly immunoreactive. The latter included 5 epithelial and 4 biophasic mesotheliomas and 1 spindle cell mesotheliomas; the immunoreaction was confined to scattered single cells, and the staining pattern was readily discernible from that of adenocarcinomas. Forty of 43 mesotheliomas were strongly immunoreactive with the broad-spectrum anti-keratin antibody, whereas 8/10 metastatic pulmonary adenocarcinomas showed focal and rather weak staining. Seven of 10 metastatic adenocarcinomas were immunoreactive to anti-CEA antibody, while only 15/43 mesotheliomas displayed weak immunoreactivity. It is concluded that monoclonal antibodies 44-3A6 and 624A12 are excellent phenotypic **markers** of metastatic pulmonary adenocarcinomas to the

pleura and thus are useful for the differential diagnosis of pleural mesotheliomas. Given conventionally fixed and processed tissues, it appears that the combined use of these monoclonal antibodies may be more effective for that differential diagnosis than anti-CEA and anti-keratin antibodies.

L27 ANSWER 28 OF 29 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 86027898 MEDLINE
DOCUMENT NUMBER: 86027898
TITLE: Immunohistochemical analysis of human pulmonary **carcinomas** using monoclonal antibody 44-3A6.
AUTHOR: Lee I; **Radosevich J A**; Ma Y X; Combs S G; Rosen S T; Gould V E
SOURCE: CANCER RESEARCH, (1985 Nov) 45 (11 Pt 2) 5813-7. Journal code: CNF. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 198602
AB A monoclonal antibody, 44-3A6, was raised against the human pulmonary adenocarcinoma cell line A549. This antibody recognizes a protein antigen at the cell surface, which is preserved after formalin fixation and paraffin embedding. Immunohistochemical staining of lung tissue with this antibody revealed diffuse immunoreactivity of type II pneumocytes. Bronchial epithelial cells were also focally immunoreactive. Immunostaining of various bronchopulmonary **carcinomas** demonstrated characteristic patterns of reactivity. All of the 42 adenocarcinomas and 18 **carcinoids** were strongly immunoreactive either diffusely or focally. The immunoreaction occurred at the cell membrane and/or in the cytoplasm. None of the 39 squamous cell **carcinomas**, 12 bronchioloalveolar **carcinomas**, and 30 small cell neuroendocrine **carcinomas** was immunostained. Ten intermediate cell neuroendocrine **carcinomas** and 8 well-differentiated neuroendocrine **carcinomas** were relatively weakly immunoreactive, while 7 and 2 of them were negative. Six adenosquamous **carcinomas** were focally positive in glandular and "basaloid" areas, whereas squamous areas were negative. Twenty-one large cell **carcinomas** were focally immunoreactive, while 6 were negative. It appears that MCA 44-3A6 is an effective **marker** for certain features of "glandular" differentiation, which may be present even in **tumors** lacking obvious glands, and that it may be useful for the differential diagnosis of various bronchopulmonary **carcinomas**.

L27 ANSWER 29 OF 29 LIFESCI COPYRIGHT 1999 CSA
ACCESSION NUMBER: 85:28067 LIFESCI